VOLUME 37 JUNE 1959 NUMBER 6

Canadian Journal of Chemistry

Editor: LÉO MARION

Associate Editors:

HERBERT C. BROWN, *Purdue University*
A. R. GORDON, *University of Toronto*
C. B. PURVES, *McGill University*
SIR ERIC RIDEAL, *Imperial College, University of London*
J. W. T. SPINKS, *University of Saskatchewan*
E. W. R. STEACIE, *National Research Council of Canada*
H. G. THODE, *McMaster University*
A. E. VAN ARKEL, *University of Leiden*

Published by THE NATIONAL RESEARCH COUNCIL

OTTAWA CANADA

Canadian Journal of Chemistry

Under the authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research, the National Research Council issues THE CANADIAN JOURNAL OF CHEMISTRY and five other journals devoted to the publication, in English or French, of the results of original scientific research. Matters of general policy concerning these journals are the responsibility of a joint Editorial Board consisting of: members representing the National Research Council of Canada; the Editors of the Journals; and members representing the Royal Society of Canada and four other scientific societies.

The Chemical Institute of Canada has chosen the Canadian Journal of Chemistry as its medium of publication for scientific papers.

EDITORIAL BOARD

Representatives of the National Research Council

I. McT. Cowan, *University of British Columbia*
A. Gauthier, *University of Montreal*
H. G. Thode (Chairman), *McMaster University*
D. L. Thomson, *McGill University*

Editors of the Journals

D. L. Bailey, *University of Toronto*
T. W. M. Cameron, *Macdonald College*
H. E. Duckworth, *McMaster University*
K. A. C. Elliott, *Montreal Neurological Institute*
Léo Marion, *National Research Council*
R. G. E. Murray, *University of Western Ontario*

Representatives of Societies

D. L. Bailey, *University of Toronto*
Royal Society of Canada
T. W. M. Cameron, *Macdonald College*
Royal Society of Canada
H. E. Duckworth, *McMaster University*
Royal Society of Canada
Canadian Association of Physicists
K. A. C. Elliott, *Montreal Neurological Institute*
Canadian Physiological Society
P. R. Gendron, *University of Ottawa*
Chemical Institute of Canada
R. G. E. Murray, *University of Western Ontario*
Canadian Society of Microbiologists
T. Thorvaldson, *University of Saskatchewan*, Royal Society of Canada

Ex officio

Léo Marion (Editor-in-Chief), *National Research Council*
J. B. Marshall (Administration and Awards), *National Research Council*

Manuscripts for publication should be submitted to Dr. Léo Marion, Editor-in-Chief, Canadian Journal of Chemistry, National Research Council, Ottawa 2, Canada.
(For instructions on preparation of copy, see **Notes to Contributors** (inside back cover).)

Proof, correspondence concerning proof, and orders for reprints should be sent to the Manager, Editorial Office (Research Journals), Division of Administration and Awards, National Research Council, Ottawa 2, Canada.

Subscriptions, renewals, requests for single or back numbers, and all remittances should be sent to Division of Administration and Awards, National Research Council, Ottawa 2, Canada. Remittances should be made payable to the Receiver General of Canada, credit National Research Council.

The journals published, frequency of publication, and prices are:

Canadian Journal of Biochemistry and Physiology	Monthly	$9.00 a year
Canadian Journal of Botany	Bimonthly	$6.00 a year
Canadian Journal of Chemistry	Monthly	$12.00 a year
Canadian Journal of Microbiology	Bimonthly	$6.00 a year
Canadian Journal of Physics	Monthly	$9.00 a year
Canadian Journal of Zoology	Bimonthly	$5.00 a year

The price of regular single numbers of all journals is $2.00.

Canadian Journal of Chemistry

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOLUME 37 JUNE 1959 NUMBER 6

STERIC INHIBITION OF RESONANCE

IV. FURTHER SPECTROSCOPIC STUDIES OF 9-NITROANTHRACENE AND 9,10-DINITROANTHRACENE[1]

JAMES TROTTER[2]

ABSTRACT

The frequencies of the NO_2 symmetrical stretching vibrations, and the ultraviolet absorption spectra, of solutions of 9-nitroanthracene and 9,10-dinitroanthracene have been measured and correlated with the deviations from coplanarity and resultant resonance inhibition.

INTRODUCTION

Detailed X-ray analyses of the crystal and molecular structures of 9-nitroanthracene and 9,10-dinitroanthracene have shown that the nitro groups are twisted* out of the planes of the aromatic rings due to the steric effects of neighboring carbon and hydrogen atoms (1, 2, 3). The variations of the characteristic vibration frequencies of the nitro group indicate resultant decreases in the resonance interactions between the groups and the aromatic π-electrons (4), and further detailed examination of the bond lengths in these molecules suggests that this resonance is almost completely inhibited in 9-nitroanthracene in the solid state, where the nitro group is rotated about the C–N bond 85° from the coplanar position, but that in crystals of the 9,10-dinitro derivative, where the twists are 64°, there is a small but measurable resonance interaction (5).

Calculations by molecular orbital methods (6) have shown that the energy of resonance interaction (E_R) between the nitro group and the aromatic rings in this type of molecule varies as $\cos^2\theta$, where θ is the angle between the planes of the rings and the nitro group. The most stable state of the molecules is determined not only by this resonance energy, but also by the van der Waals attractive and electronic repulsive forces (energy E_v) between the oxygen atoms and neighboring carbon and hydrogen atoms. There is a minimum in the E_v curve at about $\theta = 70°$, the angle corresponding to oxygen–carbon and oxygen–hydrogen separations equal to the sums of the corresponding van der Waals radii (1.7 Å for carbon, 1.4 Å for oxygen, 1.0–1.2 Å for hydrogen). Combination of resonance and van der Waals energies gives a curve with a minimum at about $\theta = 65°$, and the measured deviation from coplanarity in crystals of 9,10-dinitroanthracene (64°) is in excellent agreement with this calculated value. The most stable configuration for an isolated molecule of 9-nitroanthracene should have the nitro group twisted 64° out

[1]*Manuscript received February 3, 1959.*
Contribution from the Division of Pure Physics, National Research Council, Ottawa, Canada.
Issued as N.R.C. No. 5160.

[2]*National Research Council Postdoctorate Fellow.*

**The deviation from coplanarity has been called "twist", rather than "tilt" as in previous communications, since a referee has pointed out that "tilt" tends to suggest a deviation which involves an inclination of the* $C–NO_2$ *axis from the plane of the aromatic ring system, while the displacement in the molecules studied involves of course a torsional motion of the nitro group about the* $C–NO_2$ *axis.*

of the anthracene plane, but in the solid state crystal forces apparently result in minimum energy when $\theta = 85°$.

In solution the nitro group twists should be the same in both molecules, 64°, but probably with some oscillation of the groups through $\theta = 90°$, since the potential barrier is only about $1\frac{1}{2}$ kcal/mole (for each nitro group). The angles measured by X-ray diffraction of the crystals have been correlated very nicely with the frequencies of the NO_2 symmetrical stretching vibrations in the solid state, and these frequencies have now been measured in solution to investigate the postulation of equal twists in both molecules. The ultraviolet spectra have also been measured for correlation with the resonance inhibition.

EXPERIMENTAL

The frequencies of the NO_2 symmetrical stretching vibrations of 9-nitroanthracene and 9,10-dinitroanthracene in carbon tetrachloride solution were measured with a Perkin–Elmer model 21 infrared spectrophotometer with a rock salt prism.

The ultraviolet spectrum of 9-nitroanthracene in ethanol solution was measured with a Beckmann D.U. spectrophotometer. Since 9,10-dinitroanthracene is only very sparingly soluble in ethanol, a chloroform–ethanol mixture was used as solvent in measuring its spectrum.

DISCUSSION

The frequencies of the NO_2 symmetrical stretching vibrations in crystals of 9-nitroanthracene and 9,10-dinitroanthracene are 1374 cm^{-1} and 1367 cm^{-1} respectively (4), corresponding to nitro group twists of 85° and 64° (Table I). The corresponding frequencies in carbon tetrachloride solution are 1372 cm^{-1} for both molecules, so that the

TABLE I

NO_2 symmetrical stretching frequencies and nitro group twists

		9-Nitroanthracene	9,10-Dinitroanthracene
Crystals	NO_2 frequency	1374 cm^{-1}	1367 cm^{-1}
	Measured twist	85°	64°
Solutions	NO_2 frequency	1372 cm^{-1}	1372 cm^{-1}
	Deduced twist	64° → 90°	64° → 90°

twists are the same in both molecules in solution. The frequencies in solution cannot however be compared quantitatively with those in the solid state due to the effect of crystal forces, which generally make vibration frequencies a few wave numbers lower than the corresponding frequencies in solution. All that can be deduced is that the twists in both molecules in solution are between 64° and 90°, the value in the most stable configuration possibly depending not only on resonance and van der Waals energies (which would suggest $\theta = 64°$) but also on the effects of solvent–solute interactions.

The positions and intensities of the ultraviolet absorption bands of anthracene (7), 9-nitroanthracene, and 9,10-dinitroanthracene in ethanol solution are listed in Table II. In Table III the frequency shifts of the anthracene absorption maxima resulting from substitution are compared for the 9-cyano- (7), 9-nitro-, and 9,10-dinitro-derivatives. In 9-cyanoanthracene there is no possibility of resonance inhibition due to steric effects, and the cyano group causes a considerable displacement to longer wavelengths of the

TABLE II

Ultraviolet absorption spectra (wavelengths in mμ)

Anthracene		9-Nitroanthracene		9,10-Dinitroanthracene	
λ_{max}	log ϵ	λ_{max}	log ϵ	λ_{max}	log ϵ
324	3.43	331	3.42	335	3.34
339	3.72	347	3.61	352	3.50
356	3.90	363	3.66	367	3.62
375	3.88	382	3.58	387	3.58

TABLE III

Frequency shifts of anthracene absorption maxima

	Maximum	Frequency, cm^{-1}	Shift from anthracene, cm^{-1}
9-Cyanoanthracene	*E*	28,900	1990
	F	27,510	1960
	G	26,180	1880
	H	24,180	1830
9-Nitroanthracene	*E*	30,210	680
	F	28,820	650
	G	27,550	510
	H	26,180	460
9,10-Dinitroanthracene	*E*	29,850	1040
	F	28,410	1060
	G	27,250	810
	H	25,840	800

E-, *F*-, *G*-, *H*-bands. The corresponding shifts in the 9-nitro derivative are only[1] of about one quarter the magnitude, and those in the 9,10-dinitro about half, of the shifts in the cyano derivative. Although the cyano and nitro derivatives cannot be directly compared, these figures suggest that resonance interaction between a nitro group and the anthracene rings in 9-nitroanthracene and 9,10-dinitroanthracene is about one quarter of the interaction which would be expected in completely planar models. The resonance interaction estimated from the $\cos^2\theta$ relationship is about one fifth ($\cos^2 64°$) that in completely planar molecules, in reasonable agreement with the value deduced from the ultraviolet spectra. The intensities of the bands in the nitro derivatives are significantly lower than in anthracene, again supporting resonance inhibition.

REFERENCES

1. TROTTER, J. Acta Cryst. **11**, 564 (1958).
2. TROTTER, J. Acta Cryst. **12**, 232 (1959).
3. TROTTER, J. Acta Cryst. **12**, 237 (1959).
4. TROTTER, J. Can. J. Chem. **37**, 351 (1959).
5. TROTTER, J. Can. J. Chem. **37**, 825 (1959).
6. TROTTER, J. Can. J. Chem. **37**, 905 (1959).
7. JONES, R. N. Chem. Revs. **41**, 353 (1947).

A MANNAN PRODUCED BY BACILLUS POLYMYXA[1]

D. H. BALL[2] AND G. A. ADAMS

ABSTRACT

During the fermentation of glucose, sucrose, or lactose by *Bacillus polymyxa*, a mixture of polysaccharides is formed, one of which has been isolated by mild procedures and shown to contain mannose as the only sugar together with approximately 13% protein. Acid hydrolysis of the methylated mannan gave 2,3,4,6-tetra-*O*-methyl-D-mannose, 2,4,6-tri-*O*-methyl-D-mannose, 3,4,6-tri-*O*-methyl-D-mannose, and 3,4-di-*O*-methyl-D-mannose in the approximate ratio 2:1:1:2. Periodate oxidation results are in agreement with the methylation data and indicate a molecular weight of approximately 3000.

Bacillus polymyxa C3-2* has been studied previously in connection with its ability to ferment carbohydrates to butane-2,3-diol and this organism is of especial interest because it can utilize starch directly (1). The "polymyxins", a series of closely related antibiotics with polypeptide structures, are also produced by various strains of *B. polymyxa* (2). Polysaccharides are formed by these organisms under certain growth conditions and Misaki and co-workers have made a detailed study of the production of polysaccharides by *B. polymyxa* var. *lactoviscosus* (3). This strain, grown on lactose, produced a polysaccharide containing L-rhamnose and D-galactose in the approximate ratio of 2:1. When *B. polymyxa* C3-2 is grown with sucrose as the carbon source, a fructan is formed, presumably by a transfructosidation because this polysaccharide does not occur if glucose or lactose is used. The structure of the fructan was established by Murphy (4) and a preliminary communication reported the isolation of a mannan (5) produced by the same organism. The present paper describes the isolation of this mannan by a mild procedure and investigation of its structure.

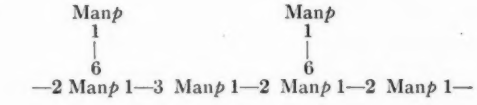
B. polymyxa C3-2 was grown on a buffered yeast extract – salts medium with glucose, sucrose, or lactose as the carbon source. A mannan was isolated by the following procedure which was considered unlikely to cause depolymerization or to affect the reducing end group. A mannose-containing polysaccharide fraction was obtained from the cell-free medium by precipitation with ethanol and this material was dissolved in water to give a highly viscous solution. Addition of aqueous Cetavlon (cetyltrimethylammonium bromide) (6, 7) to this solution precipitated the cetyltrimethylammonium salt of an acidic polysaccharide and greatly decreased the viscosity of the residual solution. Addition of sodium borate solution to the latter gave an insoluble cetyltrimethylammonium salt of a polysaccharide–borate complex (8, 9). This precipitate was dissolved in cold 0.1 *N* hydrochloric acid and the free polysaccharide was precipitated by ethanol. It was then redissolved in water and the solution was deionized on columns of ion-exchange resins and lyophilized. This material was very hygroscopic, had $[\alpha]_D$ +82°, and migrated as a single sharp peak on electrophoresis in borate buffer. Mannose was the only sugar present in an acid hydrolyzate. Several amino acids were observed chromatographically after hydrolysis with 6 *N* hydrochloric acid, and nitrogen analysis and a mannose estimation indicated that the preparation contained approximately 13% protein. Methylation of the mannan with methyl sulphate and sodium hydroxide and with silver oxide and methyl iodide in dimethylformamide (10) gave a product which could not be fractionated with

[1]*Manuscript received January 19, 1959.*
Contribution from the Division of Applied Biology, National Research Council, Ottawa, Canada.
Issued as N.R.C. No. 5159.
[2]*National Research Council of Canada Postdoctorate Fellow 1956–1958.*
**From the culture collection of the Division of Applied Biology, National Research Council of Canada, Ottawa.*

chloroform – light petroleum. The methylated mannan, which showed no hydroxyl absorption in the infrared, was hydrolyzed with formic acid and the neutralized hydrolyzate was fractionated on thick filter paper into di-, tri-, and tetra-*O*-methyl-D-mannose fractions which were present in approximately equal proportions. The syrupy tetra-*O*-methyl-D-mannose was oxidized with bromine water and converted to the crystalline phenylhydrazide of 2,3,4,6-tetra-*O*-methyl-D-mannonic acid. The trimethyl fraction was separated by filter paper electrophoresis into crystalline 3,4,6-tri-*O*-methyl-D-mannose and crystalline 2,4,6-tri-*O*-methyl-D-mannose, present in approximately equal proportions. The dimethyl fraction crystallized and was identified as 3,4-di-*O*-methyl-D-mannose.

On periodate oxidation, the mannan consumed 1.31 mole periodate and liberated 0.46 mole acid per anhydro hexose unit (assuming the presence of 13% protein not attacked by periodate). The oxidized polysaccharide was reduced with potassium borohydride and hydrolyzed. Mannose and glycerol, but not erythritol, were detected in the hydrolyzate indicating the absence in the original polysaccharide of mannose units linked through positions 1 and 4 only (11). The formaldehyde liberated during periodate oxidation at pH 8 was measured by the chromotropic acid method (12) and the results are shown in Fig. 1. The occurrence of overoxidation indicated that the predominant linkage was not 1,6 (13). After reduction of the polysaccharide with potassium borohydride, overoxidation was not observed (Fig. 2), indicating that the reducing end group was linked through position 2 (14). Calculation of the molecular weight from these results (14, 15) gave a value of 2850 and, allowing for 13% protein, this gives an average of 15 mannose units per molecule. The value of 2850 would be minimal because some terminal $1 \rightarrow 2$ linked units may be joined through C_6 position and would not give rise to formaldehyde on periodate oxidation.

These results indicate that the mannan is very similar in structure to that obtained by autolysis of yeast which gives identical methylation results (16, 17, 18). The slime polysaccharide produced by *Saccharomyces rouxii* appears to differ in containing a small proportion only of 1,3 linkages (19). On the basis of methylation data and periodate oxidation, the linkages found to be present in the *B. polymyxa* mannan are shown in the following structure.



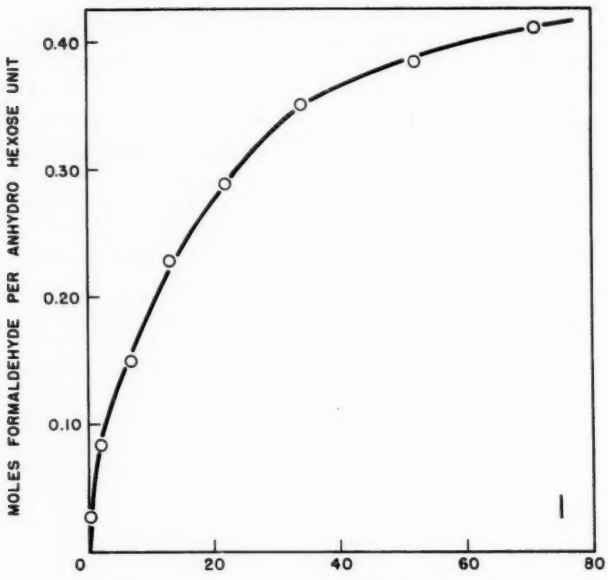
Haworth *et al.* (17) have proposed three possible repeating units for yeast mannan, one of which is the same as shown above.

EXPERIMENTAL

Solutions were concentrated under reduced pressure at 40° C. Optical rotations were determined in water at 23° ± 3° C unless otherwise stated. Paper chromatography was carried out by the descending method (20) on Whatman No. 1 filter paper using the following solvent systems: (*a*) butan-1-ol–ethanol–water (3:1:1), (*b*) butan-1-ol–pyridine–water (19:3:3), and (*c*) methyl ethyl ketone saturated with water. Sugars were located on chromatograms either by the *p*-anisidine hydrochloride spray (21) or by the silver nitrate and alkali sprays of Trevelyan *et al.* (22).

Preparation of a Mannose-containing Polysaccharide Fraction

The organism, *B. polymyxa* C3-2, was grown on the following medium: Difco yeast extract (5 g), K_2HPO_4 (0.5 g), KH_2PO_4 (0.5 g), $MgSO_4.7H_2O$ (0.2 g), sucrose (70 g) (or



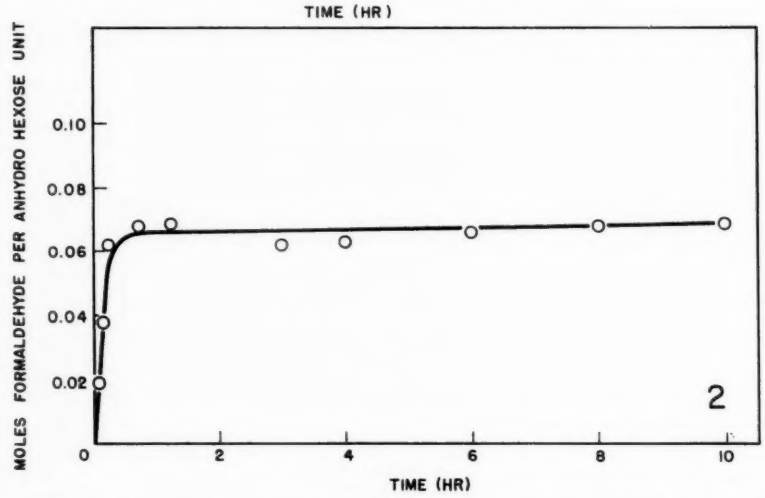


FIG. 1. Liberation of formaldehyde during periodate oxidation of the mannan at 26° C in $M/15$ phosphate buffer, pH 8.
FIG. 2. Liberation of formaldehyde during periodate oxidation of the reduced mannan at 21° C in $M/15$ phosphate buffer, pH 8.

glucose (50 g) or lactose (70 g)), water (1 l.). The medium was sterilized for 15 minutes at 15 p.s.i. in a 3-l. Fernbach flask. Sterile calcium carbonate (16 g) was added to the cooled solution. The inoculum was grown for 24 hours on the same medium (without calcium carbonate), the flask being shaken at 30° C. The inoculum (5 ml) was added to each Fernbach flask and the culture was kept at 30° C and shaken twice daily. The disappearance of sugar was followed on paper chromatograms and, in the case of glucose or

lactose, by determination of reducing sugar. Fermentations were judged to be complete after 9–10 days (sucrose), 5 days (glucose), 11 days (lactose). The media were then sterilized by heating to 90° C, filtered through cloth, and clarified on a Sharples centrifuge. The clear solution was then concentrated to 1/3 volume and the mannose-containing polysaccharide fraction was precipitated with ethanol (2/3 volume) and collected by centrifugation. Hydrolysis of a portion followed by paper-chromatographic examination indicated the presence of mannose, glucose, and galactose.

Fractionation of the Polysaccharides

To a stirred, viscous solution of the mannose-containing fraction (2.50 g) in water (500 ml) was slowly added a solution of Cetavlon (cetyltrimethylammonium bromide, 10% w/v). Precipitation occurred immediately and was complete after the addition of 10 ml. There was also a marked decrease in viscosity of the solution. The precipitate (fraction A) was collected by centrifugation, washed with water, and the washings were added to the centrifugate. Cetavlon solution (90 ml) was then added and 0.05 *M* sodium borate solution (100 ml) was run in slowly with stirring with precipitation commencing after the addition of 50 ml. The precipitate (fraction B) was collected by centrifugation and washed with water. The supernatant solution was dialyzed and poured into ethanol (4 volume). No further polysaccharide material was precipitated.

Fraction A.—The cetyltrimethylammonium salt of an acidic polysaccharide (fraction A) was not further examined.

Fraction B.—The cetyltrimethylammonium salt of a polysaccharide–borate complex (fraction B) was dissolved in cold 0.1 *N* hydrochloric acid (50 ml) and the free polysaccharide was recovered by precipitation with ethanol (300 ml). The precipitate was recovered by centrifugation, redissolved in water, and the solution was deionized by passage down columns of Amberlite resins IR 120 (H) and IR 45 (OH). The neutral effluent was lyophilized. The product (1.48 g) was hygroscopic and had $[\alpha]_D$ +82°. Found: N, 1.50%. It gave one sharp peak on electrophoresis in 0.05 *M* sodium borate with a mean mobility $\mu = 8.56 \times 10^{-8}$ cm^2 sec^{-1} v^{-1}. After hydrolysis of a portion with dilute acid, mannose was the only sugar detected on paper chromatograms. After hydrolysis with 6 *N* hydrochloric acid at 100° C for 25 hours, paper chromatograms sprayed with ninhydrin indicated the presence of several amino acids. The mannose content of the mannan as determined gravimetrically via the phenylhydrazone (23) was 87.4%.

Methylation of the Mannan

The mannan (1.9 g) was dissolved in water (15 ml) and to the vigorously stirred solution, contained in a three-necked flask under an atmosphere of nitrogen, methyl sulphate (5 ml) and 30% w/v sodium hydroxide solution (15 ml) were added simultaneously and dropwise. After 3 hours, these additions were repeated and the mixture was stirred for a further 12 hours. The solution was then heated on a hot water-bath to destroy residual methyl sulphate and remove methanol. This methylation cycle was repeated twice, water was then added to dissolve sodium sulphate and the mixture was extracted continuously with chloroform. The extracts were dried (Na_2SO_4) and concentrated to a syrup (2.6 g). This was dissolved in dry dimethylformamide (50 ml) and to the solution was added methyl iodide (20 ml) and silver oxide (20 g) (10). The mixture was shaken mechanically at room temperature in the dark for 65 hours. It was then filtered, the grey residue was washed with dimethylformamide, and to the combined filtrate and washings was added the above amounts of methyl iodide and silver oxide and the mixture was shaken for a further 24 hours. The brown-silver residues were removed by filtration, and water (100 ml) was added to the pale yellow solution. Precipitated silver salts were redissolved by

addition of potassium cyanide solution and the turbid solution was extracted eight times with chloroform, to recover the methylated product. The methylation procedure using methyl iodide and silver oxide was repeated four times. The methylated mannan was recovered as a syrup (2.0 g) which showed no hydroxyl absorption in the infrared and which could not be fractionated with chloroform – light petroleum (b.p. 65–110° C). The syrup had $[\alpha]_D$ +92° (*c*, 2.5 in $CHCl_3$); N, 1.97% and OCH_3, 38.2%.

Hydrolysis of the Methylated Mannan and Separation of the Methyl Ethers

The methylated mannan (1.5 g) was dissolved in 88% formic acid (50 ml) and the solution was heated at 90° C for 18 hours. Formic acid was then removed by evaporation, the residual syrup was taken up in 0.1 *N* hydrochloric acid, and the solution was heated at 90° C for a further 20 hours. The solution was cooled, neutralized by passage down a column of Amberlite resin IR 45 (OH), and concentrated to a syrup which was fractionated on Whatman No. 3MM filter paper, first in solvent *a* and then in solvent *c*. Three fractions were obtained: (*a*) tetra-*O*-methyl-D-mannose (0.41 g), (*b*) tri-*O*-methyl-D-mannose (0.37 g), and (*c*) di-*O*-methyl-D-mannose (0.33 g). These fractions are in a molar ratio of 1.03:1.00:0.95.

2,3,4,6-Tetra-O-methyl-D-mannose

The syrup (100 mg) which had $[\alpha]_D$ +5.6° was oxidized with bromine water. The product was isolated in the usual way and lactonized and the syrupy lactone was converted to the crystalline phenylhydrazide of 2,3,4,6-tetra-*O*-methyl-D-mannonic acid. After recrystallization from benzene, the product had m.p. 183–184° C, not depressed by admixture with an authentic sample, and $[\alpha]_D$ −25° C (*c*, 1.0 in $CHCl_3$) which were in good agreement with the reported values (24).

Tri-O-methyl-D-mannose Fraction

This fraction was separated into two components by electrophoresis on Whatman No. 3MM filter paper in 0.1 *M* sodium borate solution. The components were located by means of guide strips and eluted from the paper with cold water. The aqueous solutions were passed down short columns of Amberlite resin IR 120 (H) and evaporated to syrups which were evaporated three times from methanol to remove borate ions as methyl borate.

Fraction 1.—Fraction 1 gave a brown color with the *p*-anisidine spray and had M_G 0.12 (movement relative to glucose on a paper electrophorogram). It crystallized readily and after recrystallization from ether–*n*-hexane had m.p. 100–102° C not depressed by admixture with authentic 3,4,6-tri-*O*-methyl-D-mannose; $[\alpha]_D$ +21° → +8° (const., 20 hours). These values are in agreement with those reported for this sugar (25).

Fraction 2.—Fraction 2 gave a red color with the *p*-anisidine spray and had M_G 0.0. The syrup crystallized when seeded with authentic 2,4,6-tri-*O*-methyl-D-mannose monohydrate (26) and after recrystallization from ether–*n*-hexane had m.p. and mixed m.p. 63–65° C and $[\alpha]_D$ +17° (no mutarotation).

3,4-Di-O-methyl-D-mannose

The dimethyl mannose crystallized as the monohydrate and after two recrystallizations from ethyl acetate had m.p. 70–73° C not depressed by admixture with an authentic sample (19). $[\alpha]_D$ +17.6° (10 minutes) → +10° (const., 2 hours). Anal.: Calc. for $C_8H_{16}O_6.H_2O$: C, 42.48%; H, 8.02%. Found: C, 42.47%; H, 7.76%.

Periodate Oxidation of the Mannan

Oxidation of the mannan with 0.03 *M* sodium metaperiodate solution at room temperature was complete after 60 hours with the consumption of 1.31 mole of periodate

and the liberation of 0.46 mole of acid per anhydro hexose unit (all values given are corrected to allow for the 13% protein present). The oxidized polysaccharide was reduced with excess potassium borohydride without prior isolation. After 24 hours at room temperature, the solution was acidified with sulphuric acid and heated at 100° C for 6 hours to hydrolyze the polyol. Chromatographic examination of the deionized hydrolyzate indicated mannose and glycerol only. Erythritol was absent.

The mannan was also oxidized at 27° C with 0.03 *M* sodium metaperiodate in *M*/15 phosphate buffer, pH 8.0, and the formaldehyde liberated was determined by the chromotropic acid method (12). The results, shown in Fig. 1, indicate that extensive overoxidation occurred. After borohydride reduction of the polysaccharide, overoxidation was not observed (Fig. 2). Because only 1 mole of formaldehyde is liberated per mole of polymer, these results give an average molecular weight of 2850 and, allowing for 13% protein, this gives an average of 15 mannose units per molecule.

ACKNOWLEDGMENTS

The authors are grateful to Miss M. T. Clement for assistance with the fermentations, to Professor J. K. N. Jones for samples of 2,4,6- and 3,4,6-tri-*O*-methyl-D-mannose, and to Dr. P. A. J. Gorin for a sample of 3,4-di-*O*-methyl-D-mannose.

REFERENCES

1. LEDINGHAM, G. A. and NEISH, A. C. *In* Industrial fermentations. Vol. II. *Edited by* L. A. Underkofler and R. J. Hickey. Chemical Pub. Co., Inc., N.Y. 1954. p. 27.
2. LONG, P. H. *et al.* Ann. N. Y. Acad. Sci. **51**, 853–1000 (1949).
3. MISAKI, A. *et al.* J. Fermentation Technol. (Japan), **32**, 147, 191, 311, 315, 341 (1954); **36**, 25, 30, 44, 176, 181, 266, 271 (1958).
4. MURPHY, D. Can. J. Chem. **30**, 872 (1952).
5. MURPHY, D., BISHOP, C. T., and ADAMS, G. A. Can. J. Biochem. and Physiol. **34**, 1271 (1956).
6. JONES, A. S. Biochim. et Biophys. Acta, **10**, 607 (1953).
7. BERA, B. C., FOSTER, A. B., and STACEY, M. J. Chem. Soc. 3788 (1955).
8. BARKER, S. A., STACEY, M., and ZWEIFEL, G. Chem. & Ind. 330 (1957).
9. PALMSTIERNA, H., SCOTT, J. E., and GARDELL, S. Acta Chem. Scand. **11**, 1792 (1957).
10. KUHN, R., TRISCHMANN, H., and LOW, I. Angew. Chem. **67**, 32 (1955).
11. HAMILTON, J. K. and SMITH, F. J. Am. Chem. Soc. **78**, 5907 (1956).
12. O'DEA, J. F. and GIBBONS, R. A. Biochem. J. **55**, 580 (1953).
13. HOUGH, L. and PERRY, M. B. Chem. & Ind. 768 (1956).
14. HOUGH, L., WOODS, B. M., and PERRY, M. B. Chem. & Ind. 1100 (1957).
15. UNRAU, A. M. and SMITH, F. Chem. & Ind. 330 (1957).
16. HAWORTH, W. N., HIRST, E. L., and ISHERWOOD, F. A. J. Chem. Soc. 784 (1937).
17. HAWORTH, W. N., HEATH, R. L., and PEAT, S. J. Chem. Soc. 833 (1941).
18. CIFONELLI, J. A. and SMITH, F. J. Am. Chem. Soc. **77**, 5682 (1955).
19. GORIN, P. A. J. and PERLIN, A. S. Can. J. Chem. **34**, 1796 (1956).
20. PARTRIDGE, S. M. Biochem. J. **42**, 238 (1948).
21. HOUGH, L., JONES, J. K. N., and WADMAN, W. H. J. Chem. Soc. 1702 (1950).
22. TREVELYAN, W. E., PROCTOR, D. P., and HARRISON, J. S. Nature, **166**, 444 (1950).
23. HIRST, E. L., JONES, J. K. N., and WOODS, E. A. J. Chem. Soc. 1048 (1947).
24. DREW, H. D. K., GOODYEAR, E. H., and HAWORTH, W. N. J. Chem. Soc. **130**, 1237 (1927).
25. BATT, H. G., HAWORTH, W. N., and HIRST, E. W. J. Chem. Soc. 1395 (1930).
26. PRENTICE, N., CUENDET, L. S., and SMITH, F. J. Am. Chem. Soc. **78**, 4439 (1956).

5,6-DIDEOXY-L-ARABINO-HEXOSE (5-DEOXY-5-*C*-METHYL-L-ARABINOSE)[1]

D. J. BALL, A. E. FLOOD, AND J. K. N. JONES

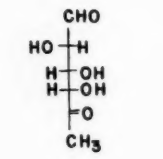
ABSTRACT

A synthesis of 5,6-dideoxy-L-*arabino*-hexose (II) from methyl β-D-galactofuranoside is described.

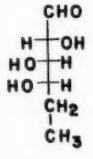
INTRODUCTION

The isolation of 6-deoxy-5-keto-D-*arabino*-hexose (6-deoxy-D-*arabino*-hexos-5-ulose) (I) from hygromycin has recently been described (1). This substance was converted by reduction to 5,6-dideoxy-D-*arabino*-hexose, which was then characterized as its dithioacetal (1). The purpose of this communication is to describe the synthesis of its optical isomer, 5,6-dideoxy-L-*arabino*-hexose (II). The elimination of tosyloxy (tolyl-*p*-sulphonyloxy) groups situated on vicinal primary and secondary alcoholic groups by heating the esters with sodium iodide is a general reaction (2) which has been observed in the hexitol (3), hexose (4), and inositol series (5).

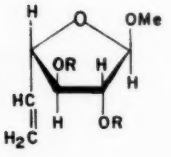
Crystalline derivatives of ethyl α- and β-D-galactofuranosides are known, but their preparation is tedious. It was observed that an impure syrupy preparation of methyl β-D-galactofuranoside (6) which had been converted to the 2,3,5,6-tetra-toluene-*p*-sulphonate gave, when heated with a solution of sodium iodide in acetone, a crystalline methyl 5,6-didehydroxy-2,3-di-*O*-tosyl-α-L-*arabino*-hexoside (III; R = tosyl = tolyl-*p*-sulphonyl).



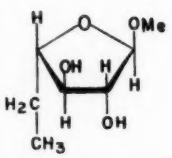
(I)



(II)



(III)



(IV)

Removal of the tosyl groups by reductive saponification yielded crystalline methyl 5,6-didehydroxy-α-L-*arabino*-hexoside (III; R = H) which, when it was reduced (Raney nickel catalyst), furnished 5,6-dideoxy-α-L-*arabino*-hexoside (IV). This substance (IV) was also produced when the unsaturated tosyl derivative of III was reduced by boiling its solution in ethanol in the presence of Raney nickel, followed by reductive saponification of the tosyl groups.

[1]*Manuscript received September 30, 1958.*
Contribution from the Department of Organic Chemistry, Queen's University, Kingston, Ontario.

Can. J. Chem. Vol. 37 (1959)

Tosylation of IV yielded a product indistinguishable from the substance III (R = H) produced by reduction of III (R = tosyl). This experiment proved that inversion of hydroxyl groups had not occurred during removal of the tolyl-*p*-sulphonyl groups in the preparation of III (R = H) and IV.

Both III (R = H) and IV were oxidized by aqueous sodium metaperiodate and yielded significant quantities of iodoform when the oxidized products were allowed to stand in iodine solution. When IV was shaken with ethanethiol and concentrated hydrochloric acid it yielded the diethyl dithioacetal of II. The D-isomer of this derivative was isolated from hygromycin.

When III (R = tosyl) was shaken with silver nitrate and iodine in acetonitrile solution (7), a crystalline deoxy-iodonitrate of III (R = tosyl) was produced. The location of the iodine group in this new product is undecided, as is the decision as to whether the substance is a derivative of L-altrose or of D-galactose.

EXPERIMENTAL

Solutions were evaporated under reduced pressure; optical rotations were determined at 20°±2° C (unless otherwise stated).

Synthesis of Methyl 5,6-Didehydroxy-2,3-di-O-tolyl-p-sulphonyl-α-L-arabino-hexoside (III; R = Tosyl)

Crude methyl β-D-galactofuranoside (from 50 g of D-galactose (6)) was dissolved in pyridine (500 ml), and toluene-*p*-sulphonyl chloride (250 g) was added to the solution. After 20 hours, the solution was poured (with stirring) onto crushed ice. The syrupy tosyl derivatives were isolated in the usual way. The syrup, without further purification, was heated in acetone solution (500 ml) with sodium iodide (70 g) under reflux. The solution rapidly became brown because of the separation of iodine, and sodium toluene-*p*-sulphonate precipitated. The mixture was filtered at intervals to minimize uneven boiling. (Yield of the sodium salt of toluene-*p*-sulphonic acid, 36.5 g.)

The acetone was removed and the residue was extracted with chloroform. The extracts were washed successively with a solution of sodium thiosulphate and then with water. The chloroform extract was dried ($MgSO_4$) and evaporated to a syrup which rapidly crystallized. The crystals (32 g) were recrystallized from methanol, m.p. 109–110° C, $[\alpha]_D$ −62°±2° (*c*, 0.5 in chloroform). Anal. Calc. for $C_{21}H_{24}O_8S_2$: C, 53.8; H, 5.1; S, 13.7; OMe, 6.5. Found: C, 53.7; H, 5.5; S, 14.7; OMe, 6.5.

Methyl 5,6-Didehydroxy-α-L-arabino-hexoside (III; R = H)

The di-*O*-tosyl derivative (above) (1 g) was dissolved in ethanol (100 ml), and sodium amalgam (100 g of 3%) was added portionwise with stirring. During the addition, water (150 ml) was added at such a rate that precipitation of the tosyl derivative was avoided. After 12 hours, the solution was made neutral with sulphuric acid and evaporated to dryness. The solid residue was extracted exhaustively with acetone and the extracts were evaporated. The crude crystalline residue (0.21 g) was volatile at 100° C and accordingly it was purified by sublimation at 12 mm/100° C. The product, after sublimation, had m.p. 60° C, $[\alpha]_D^{23}$ −110°±2° (*c*, 0.67), and was analytically pure. The substance moved at 2.4 times the speed of rhamnose in *n*-butyl alcohol – ethanol – water (9:2:2) solvent, and gave a yellow coloration when it was heated with *p*-anisidine hydrochloride. Anal. Calc. for $C_7H_{12}O_4$: C, 52.5; H, 7.6; OMe, 19.4. Found: C, 52.4; H, 7.8; OMe, 22.3.

Methyl 5,6-Dideoxy-α-L-arabino-hexoside (IV) (*Methyl 5-Deoxy-5-C-methyl-α-L-arabinoside*)

The unsaturated arabinoside derivative (above, 89 mg) was dissolved in methanol and hydrogenated at 20° in the presence of a Raney nickel catalyst (0.5 g). After 20 minutes, the hydrogen uptake (53.4 ml at S.T.P.) was constant. The solution was filtered and the filtrate evaporated. The product was purified by sublimation at 16 mm/100° C. The product had m.p. 63° C and $[\alpha]_D$ 126°±6° (*c*, 0.18). Anal. Calc. for $C_7H_{14}O_4$: C, 51.8; H, 8.7; OMe, 19.1. Found: C, 51.9; H, 8.7; OMe, 19.8.

Both this compound and the unsaturated glycoside were oxidized by sodium metaperiodate solution, with the consumption of more than one mole of reagent, per mole, and with the formation of iodine and iodoform. No formic acid was detected.

Methyl 5,6-Dideoxy-2,3-di-O-tosyl-α-L-arabino-hexoside

The unsaturated tosyl derivative (III; R = tosyl) (116 mg) was dissolved in ethanol (20 ml) and boiled under reflux in the presence of Raney nickel catalyst (0.5 g) for 4 hours. The odor of acetaldehyde was detectable during the reaction. The solution was cooled and filtered, and the filtrate evaporated to a crystalline solid which was purified by recrystallization from ethanol. The product had m.p. 93–94° C, $[\alpha]_D$ −61° (*c*, 2.23 in chloroform). A product which had the same properties was produced when methyl 5,6-dideoxy-α-L-*arabino*-hexoside (IV; 0.1 g) was allowed to react with toluene-*p*-sulphonyl chloride (0.3 g) in pyridine (5 ml) solution overnight. The substance, which was isolated after the pyridine solution had been poured onto ice, weighed 0.21 g and had m.p. 93–94° C, not depressed on admixture with an authentic specimen. Anal. Calc. for $C_{21}H_{26}O_8S_2$: C, 53.6; H, 5.6; S, 13.6; OMe, 6.6. Found: C, 53.8; H, 6.0; S, 12.9; OMe, 6.6.

5,6-Didehydroxy-L-arabino-hexose Diethyl Dithioacetal

Methyl 5,6-dideoxy-α-L-*arabino*-hexoside (0.1 g) was shaken with ethanethiol (0.5 ml) and 6 *N* hydrochloric acid (0.5 ml) for 5 hours. The mixture was twice extracted with chloroform (2-ml portions) and the extracts were washed with sodium bicarbonate solution and then dried ($MgSO_4$). Removal of the chloroform left a syrupy diethyl dithioacetal which crystallized. It was recrystallized from water and had m.p. 110°. Anal. Calc. for $C_{10}H_{22}O_3S_2$: C, 47.3; H, 8.7; S, 25.2. Found: C, 47.4; H, 8.8; S, 24.8.

Iodonitration of Methyl 5,6-Didehydroxy-2,3-di-O-tolyl-p-sulphonyl-α-L-arabino-hexoside

Methyl 5,6-didehydroxy-2,3-di-*O*-tolyl-*p*-sulphonyl-α-L-*arabino*-hexoside (2.34 g; 5 millimole) was dissolved in acetonitrile (50 ml), and to the solution was added silver nitrate (1.70 g; 10 millimole) and iodine (1.27 g; 5 millimole). An immediate precipitate of silver iodide was observed. The mixture was shaken at room temperature for 7 days in a stoppered flask from which light was excluded. The mixture was then filtered and the silver iodide was washed with acetonitrile and air-dried; yield 1.22 g. (The reaction of silver nitrate with iodine to give silver iodide and iodonium nitrate would give 1.17 g of silver iodide from the above amounts of reactants.)

The filtrate was boiled under reflux for 24 hours and a light precipitate was removed by filtration. Concentration of the filtrate afforded a yellow syrup which yielded a further precipitate (probably silver nitrate) when diluted with chloroform. The mixture was again filtered and the filtrate was evaporated to a syrup which crystallized from chloroform – light petroleum (b.p. 60–80° C). Total yield 0.36 g after recrystallization from the same solvents; i.e. 11.0% calculated as the iodonitrate. The compound had

m.p. 125–126° C and $[\alpha]_D$ −49° (*c*, 5.0 in chloroform). Anal. Calc. for $C_{21}H_{24}INO_{11}S_2$: C, 38.4; H, 3.7; I, 19.3; N, 2.1; S, 9.8. Found: C, 38.4; H, 3.8; I, 18.6; N, 2.6; S, 9.5.

The infrared absorption spectrum showed a strong band at 1642 cm^{-1} which was absent from the spectrum of the parent olefinic compound and is presumably due to the nitrate group. The residual syrup (1.85 g) gave an infrared absorption spectrum with bands of the same relative intensity and therefore probably contains one or more of the other three possible iodonitrates.

A portion of the crystalline iodonitrate (0.10 g) was dissolved in hot ethanol and the solution was refluxed for 90 minutes with hydrazine hydrate (0.03 ml) (8). Excess hydrazine was destroyed by boiling the solution for a further 15 minutes with Raney nickel. The mixture was then filtered and the filtrate was concentrated to an oil which was soluble in water but not soluble in chloroform. The properties of this product indicate that nitrate and tolyl-*p*-sulphonyl groups had both been removed. It was not further examined.

The authors thank the National Research Council for a grant which made this work possible.

REFERENCES

1. MANN, R. L. and WOOLF, D. O. J. J. Am. Chem. Soc. **79**, 120 (1957).
2. TIPSON, R. S. Advances in Carbohydrate Chem. **8**, 201, 205 (1953).
3. BLADON, P. and OWEN, L. N. J. Chem. Soc. 598 (1950).
4. JONES, J. K. N. and THOMPSON, J. L. Can. J. Chem. **35**, 955 (1957).
5. ANGYAL, S. J. and GILHAM, P. T. J. Chem. Soc. 375 (1958).
6. MOWERY, D. F. and FERRANTI, G. R. J. Am. Chem. Soc. **76**, 4103 (1954).
7. MORRIS, L. J. Chem. & Ind. (London), 1291 (1958).
8. ENNOR, K. S. and HONEYMAN, J. J. Chem. Soc. 2586 (1958).

IRRADIATION OF AQUEOUS CHLORAL HYDRATE WITH Sr^{90}–Y^{90} BETA RAYS[1]

R. F. Platford and J. W. T. Spinks

ABSTRACT

Air-saturated aqueous chloral hydrate has been irradiated with beta rays and the acid yield as determined by titration has been studied as a function of dose rate, temperature, and initial concentration of the chloral hydrate.

The average lifetime of the intermediates has been found, by means of the rotating sector technique, to be about 0.5 second. Propagation and termination rate constants for the reaction have been calculated and an effort has been made to explain the results observed in terms of a free radical chain reaction.

INTRODUCTION

In 1950 Andrews and Shore (1) reported that aqueous chloral hydrate was decomposed by X rays to yield HCl and other products. The acid yield, measured conductometrically, was found to be linear with radiation dose, and to increase with increasing temperature and initial chloral hydrate concentration, but was reported to be independent of dose rate. Because of the large acid yield, these workers used the system as a sensitive radiation dosimeter.

Freeman *et al.* in 1953 (2) irradiated the same system with Co^{60} γ-rays and found essentially the same results as Andrews and Shore except that they found the acid yield to be dependent on $(\text{dose rate})^{-1/2}$. Freeman also found the radiation yield in air-free solutions to be about one fifth of that in aerated solutions.

Andrews *et al.* (3) have used an agar gel containing chloral hydrate and methyl red as a dosimeter for Van de Graaff electrons.

In the present work the decomposition of chloral hydrate by beta rays is compared with that resulting from gamma rays.

EXPERIMENTAL

Apparatus

Irradiations were carried out with a 40-mc Sr^{90}–Y^{90} medical applicator. The activity was confined to an area of 1 cm^2 and was plated on one face of an aluminum disk to the back of which was attached a rod serving as a handle. Solutions were irradiated in 10-ml pyrex beakers immersed in a thermostat, above which the source was suspended. The dose rate received by the solution was controlled by varying the separation between source and solution.

A rotating sector with a light:dark ratio of 1:2 was constructed from brass of thickness 1335 mg/cm^2. The sector was driven through a reduction drive by a 240-r.p.m. synchronous motor.

Materials

All chemicals were reagent grade or better; the chloral hydrate was Fisher U.S.P. grade. All water was distilled once from alkaline permanganate and once from acidic dichromate solution.

Procedure

The reaction was followed by titration of the acid produced on irradiation. Samples

[1]*Manuscript received February 9, 1959.*
Contribution from the Department of Chemistry, University of Saskatchewan, Saskatoon, Saskatchewan.

were titrated with *ca.* 10^{-2} *N* NaOH from a microburette which could be read to 5×10^{-4} ml. Irradiated solutions were titrated to the same color as unirradiated blanks using bromocresol purple as an indicator.

The energy absorbed on irradiation was estimated by means of the ferrous sulphate dosimeter (0.8 *N* H_2SO_4, 10^{-2} *M* NaCl, 10^{-3} *M* $(NH_4)_2SO_4.FeSO_4.6H_2O$ in air-saturated water). The *G* value (molecules of Fe^{++} oxidized per 100 ev of energy absorbed) was assumed to be 15.5 (4, 5, 6). The Fe^{+++} produced on irradiation was measured by means of its absorption peak at 304 mμ. Dose rates are expressed in rad/min, where 1 rad ≡ 100 erg/g.

Because the densities of irradiated solutions differed from that of the ferrous sulphate solution, a small correction was applied to compensate for differences in stopping power between the two solutions. The stopping power for electrons was assumed proportional to the solution density.

RESULTS

Effect of Dose Rate

The *G* value (molecules of acid produced per 100 ev of energy absorbed) was found to be dependent on dose rate. Also, because of the strong attenuation of beta rays by the solution, the dose rate varied with depth of solution and hence the *G* value was dependent on solution depth (or volume, since the cross sectional area of the solutions was constant). The acid yield vs. dose is shown as a function of dose rate for two different volumes of solution in Figs. 1 and 2. The *G* value, which is equal to 965×(acid yield)/(dose in rad), is shown in Table I as a function of dose rate, and *G* vs. (dose rate)$^{-1/2}$ is plotted in Fig. 3. For comparison, a plot of *G* vs. (dose rate)$^{-1/2}$ is shown for chloral hydrate irradiated with Co^{60} γ-rays for which attenuation of radiation by the solution was very small.

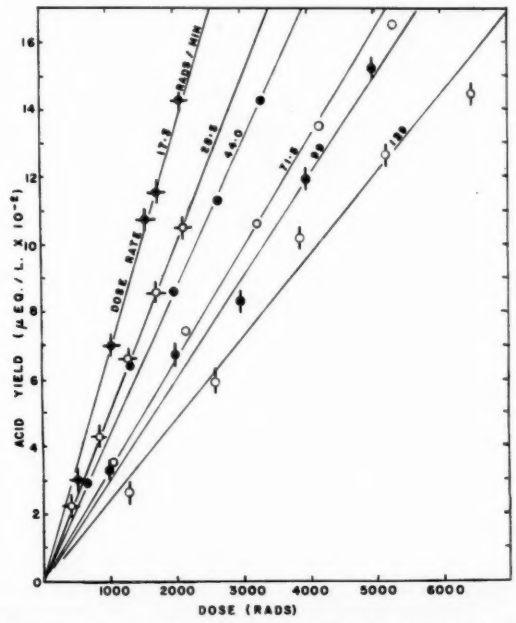


FIG. 1. Effect of dose rate on acid yield in 1 ml of a 1 *M* chloral hydrate solution at 25° C.

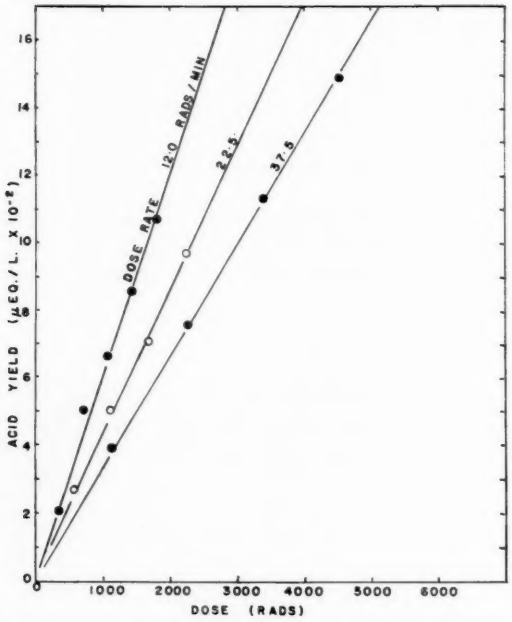


FIG. 2. Effect of dose rate on acid yield in 5 ml of a 1 *M* chloral hydrate solution at 25° C.

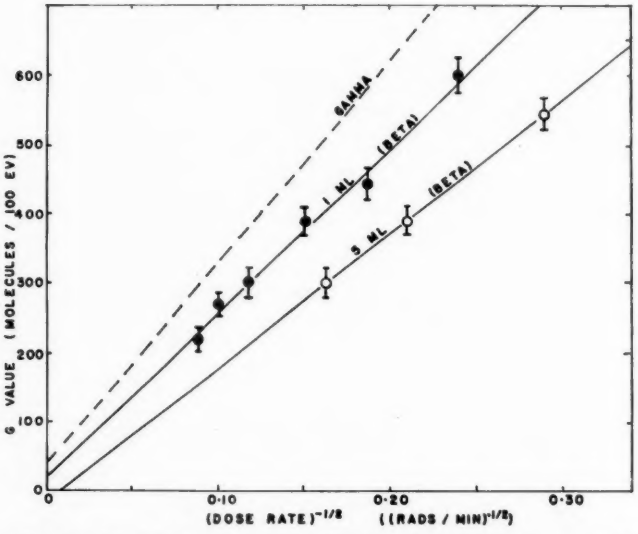


FIG. 3. Effect of dose rate on *G* value for 1 *M* chloral hydrate at 25° C.
– – – – Co^{60} gamma radiation. ———— Sr^{90}–Y^{90} beta radiation.

TABLE I

G value as a function of dose rate for 1 *M* chloral hydrate irradiated at 25° C

Dose rate (rad/min)	*G* value (molecules/100 ev)
1-ml solution (thickness = 2.7 mm)	
129 ±6	220±15
99 ±4	270±15
71.5±2.5	300±20
44.0±1	390±20
28.5±0.7	445±25
17.3±0.4	600±25
5-ml solution (thickness = 13.5 mm)	
37.5±0.8	300±15
22.5±0.4	390±20
12.0±0.4	545±20

Temperature Coefficient

The *G* value was determined at three temperatures and the results are shown in Table II. The temperature coefficient for the reaction was 3.3% per ° C at 25° C, which corresponded to an activation energy of 5.5±1 kcal/mole.

TABLE II

Effect of temperature on *G* value for 1 *M* aqueous chloral hydrate irradiated at 44.0 rad/min (1-ml solution)

Temperature (° C)	*G* value
15.5±0.2	285±20
25 ±0.2	390±20
35 ±0.2	540±20

Effect of Concentration

The results showing the dependence of *G* value on concentration are shown in Table III and in Fig. 4. Since the densities of the more concentrated solutions were so much

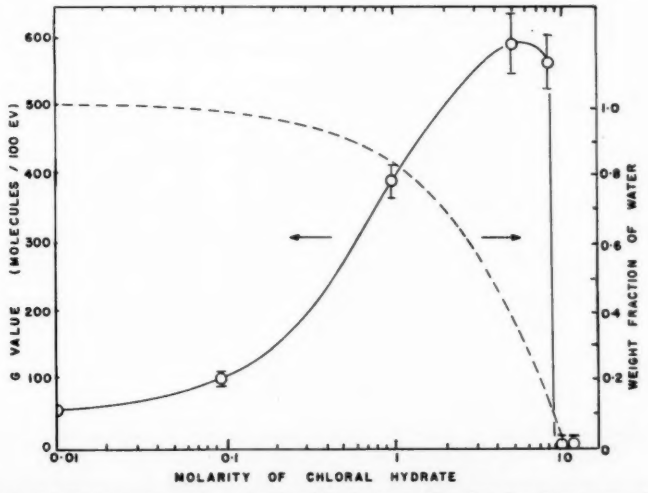


FIG. 4. Effect of initial concentration of chloral hydrate on *G* value for dose rates of 45 rad/min.

different from that of the dosimeter solution, different dose rates resulted in the two solutions, due to differences in stopping powers per unit *volume*. It was assumed that the stopping power per unit *weight* was the same in both the chloral hydrate and dosimeter solution and that the dose rate in the concentrated chloral hydrate solution was the same as that in an equal weight of dosimeter solution.

The 10 *M* solution was saturated at about 35° C and when cooled to 25° C formed a solid solution which was irradiated in this state.

TABLE III

Effect of concentration on *G* value for chloral hydrate irradiated at 25° C (1-ml solution)

Concentration	*G* value
Dose rate = 45.0 rad/min	
0.01 *M*	53±5
0.1 *M*	100±10
Dose rate = 44.0 rad/min	
1.0 *M*	390±20
5 *M*	600±40
8 *M* (satd.)	570±40
10 *M* (solid soln.)	<25
Solid chloral hydrate	<25

It will be observed that the standard deviations are larger in more concentrated solutions; this was due to a less certain titration end point in the more highly concentrated solutions.

Effect of Intermittent Irradiation

Solutions of 1 *M* chloral hydrate at 25° C were irradiated with pulsed radiation, produced by interposing a rotating sector between source and solution. The effect of pulse time on *G* value is shown in Fig. 5. The reduction in *G* value occurs when the pulse time is about equal to the mean lifetime of the reaction intermediates (7).

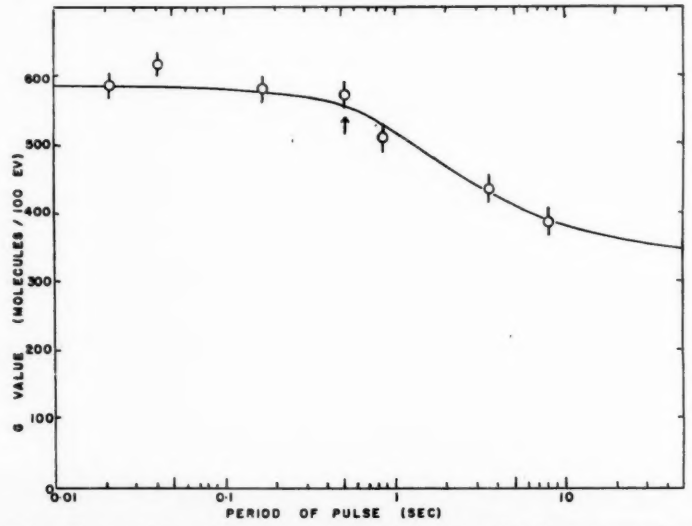


FIG. 5. Effect of intermittent radiation on *G* value. The dose rate with the sector rotating was 18 rad/min The average lifetime of the intermediates corresponds to the period of pulse shown by the arrow.

DISCUSSION

The large dose rate dependent G values indicate a chain reaction as the mechanism by which the beta radiolysis proceeds. The dependence of G value on (dose rate)$^{-1/2}$ means that a second-order recombination is responsible for removal of the chain-propagating intermediates. Lack of knowledge of reaction products, however, precluded setting up a reaction mechanism.

The dependence of G on thickness of solution irradiated was explained by assuming that the dose rate varied exponentially with solution depth and that the reaction velocity V varied with the (dose rate)$^{1/2}$ (this is tantamount to saying $G \propto$ (dose rate)$^{-1/2}$).

These conditions give:

$$V_x = KI_x^{\frac{1}{2}}$$

where V_x = reaction rate at depth x in solution,
K = a constant,
I_x = dose rate at depth x in solution,

where x is expressed in g/cm^2.

Also:

$$I_x = I_0\, e^{-\mu x},$$

where I_0 = surface dose rate,
μ = mass absorption coefficient in cm^2/g.

Now we have:

$$V_x = KI_0^{\frac{1}{2}}\, e^{-\mu x/2}.$$

The average reaction rate $\bar{V}$ in a solution of thickness t is given by:

$$\bar{V} = \frac{1}{t}\int_0^t V_x dx = \frac{1}{t}\,(2KI_0^{\frac{1}{2}}/\mu)\,(1-e^{-\mu t/2}).$$

The average dose rate $\bar{I}$ is given by:

$$\bar{I} = I_0(1-e^{-\mu t})/\mu t.$$

Therefore the G value in such a solution is given by:

$$G = \bar{V}/\bar{I} = \frac{2KI_0^{\frac{1}{2}}(1-e^{-\mu t/2})/\mu t}{I_0\,(1-e^{-\mu t})/\mu t},$$

$$G = 2K/[I_0^{\frac{1}{2}}(1+e^{-\mu t/2})].$$

Now $\mu = 6.1$ cm^2/g for solutions used here and $e^{-\mu t/2} = 0.43$ for the 1-ml solution (thickness = 0.27 g/cm^2).

Because absorption of the beta rays was complete in this thickness of solution (1.40 g/cm^2), $\mu t \to \infty$ for 5-ml solutions.

The *surface* dose rates for 1-ml and 5-ml solutions irradiated at the same *average* dose rates were found by extrapolation of a plot of dose rate vs. solution thickness to zero solution thickness. These dose rates were found to be in the ratio:

$$\frac{I_0\,(1\text{ ml})}{I_0\,(5\text{ ml})} = \frac{1}{3.1}.$$

Substitution into the expression for G value gave:

$$\frac{G\,(1\text{ ml})}{G\,(5\text{ ml})} = \frac{I_0^{\frac{1}{2}}(5\text{ ml})\,(1+e^{-\mu t/2})\,(5\text{ ml})}{I_0^{\frac{1}{2}}(1\text{ ml})\,(1+e^{-\mu t/2})\,(1\text{ ml})} = \frac{\sqrt{3.1}}{1}\,\frac{1}{1.43} = 1.23.$$

The experimental value of the above ratio was found by taking the ratios of the slopes of the 1-ml and 5-ml lines in Fig. 3. This ratio was found to be 1.20. The above explanation accounts for the observed dependence of G value on thickness of solution. The difference between G values for γ-radiation and beta radiation seen in Fig. 3 may also be due to such an effect since the gamma radiation is attenuated even less than beta radiation in a 1-ml solution of the thickness used here.

The dependence of G value on concentration shown in Fig. 4 may be explained by saying that at lower concentrations the probability of reaction of chain-initiating radicals with chloral molecules increased with chloral hydrate concentration; at high chloral hydrate concentrations the water concentration falls off rapidly and very few radicals are produced from the water per unit volume of solution.

The mean lifetime of the reaction intermediates was found to be about 0.5 second and from this figure approximate propagation and termination rate constants were calculated. It can be shown (8) that the termination rate constant k_t is given by:

$$k_t = 1/\tau^2\phi I,$$

where τ = mean lifetime of the free radical intermediates (here = 0.5 second),
ϕ = G value for radical initiation (taken to be 5 radicals/100 ev),
I = dose rate,
k_t = 2.5×10^9 l./mole-sec.

Also, it was assumed that V, the reaction velocity, was given by:

$$V = k_p\,[C](\phi I/k_t)^{\frac{1}{2}},$$

where k_p = propagation rate constant,
$[C]$ = chloral hydrate concentration.

The reaction rate $V = 2.0\times10^{-7}$ mole/l.-sec, $k_p = 2.5\times10^2$ l./mole-sec at a dose rate of 18.0 rad/min.

It would appear that no great difference exists between the beta and gamma radiolysis of aqueous chloral hydrate except for effects due to attenuation of the radiation. This is to be expected since the processes by which chain-initiating radicals are produced are the same in each case.

REFERENCES

1. Andrews, H. L. and Shore, P. A. J. Chem. Phys. **18**, 1165 (1950).
2. Freeman, G. R., Van Cleave, A. B., and Spinks, J. W. T. Can. J. Chem. **32**, 322 (1954).
3. Andrews, H. L., Murphy, R. E., and Lebrun, E. J. Rev. Sci. Instr. **28** (5), 329 (1957).
4. Hochanadel, C. J. and Ghormley, J. A. J. Chem. Phys. **21**, 880 (1953).
5. Saldick, J. and Allen, A. O. J. Chem. Phys. **22**, 438 (1954).
6. Schuler, R. H. and Allen, A. O. J. Chem. Phys. **24**, 56 (1956).
7. Dickinson, R. G. *In* The photochemistry of gases. Reinhold Publishing Corp., New York. **1941.** pp. 202–209.
8. Dainton, F. S. and Tordoff, M. Trans. Faraday Soc. **53**, 599 (1958).

SOME SPECIFIC FEATURES IN AUTOXIDATION OF METHYL LINOLEATE[1]

N. A. KHAN

ABSTRACT

A scheme of manual counter-current extraction, ingeniously devised, has facilitated quantitative isolation of initial products from autoxidized methyl linoleate under controlled conditions. A sole product of monomeric monohydroperoxide with one spatial configuration has been shown to be the outcome of interactions of oxygen molecule with methyl linoleate. The physical and chemical analyses prove this product as conjugated *cis,trans* methyl linoleate hydroperoxide. The fractionations of its derivative, hydroxystearic acid, and its scission products go to establish the formation of two positional isomers (9- and 13-) exclusively for this *cis,trans* product. Such positional isomers leave no doubt about two possibilities of attack by oxygen on the two 3-C segments in the 5-C system of methyl linoleate containing mobile π electrons. A mechanism has been proposed relevant to the experimental findings. Such initiation processes offer the missing link between neutral methyl linoleate and formation of its hydroperoxide without decomposition. These investigations, thus, emphasize the fact that the initiation processes must be distinguished from the main autoxidation reactions.

In continuation of the previous studies (9) it is necessary to describe the carefully outlined procedures for the isolation of methyl linoleate hydroperoxides. The quantitative isolation of pure hydroperoxides, formed exclusively during the initial stages of autoxidation reactions of methyl linoleate, has been successfully carried out. The purity of such products has also been established through preparation of several derivatives and relevant scission products. Infrared studies on the fatty acid esters and their hydroperoxides have revealed many clues to the mechanism of autoxidation reactions. On the basis of these data and the other experimental facts, the mechanism of the initial attack by oxygen molecule on methylene-interrupted double bonds (5-C system of methyl linoleate) has been presented.

Methyl linoleate (545 g) was freshly prepared from sesame oil acids by bromination and debromination procedures. The *trans* isomers (isolated *trans* absorbing at 10.32 μ) formed during debromination were practically removed through multiple urea complex formation under different conditions. One per cent solution of linoleate sample in methanol was each time saturated at 30° C and crops of crystals were obtained at 4° C, yielding 4.6, 5.9, 7.3, 4.2, and 2.6% of the substance taken. The solution concentration at 1% was restored by evaporation under vacuum at room temperatures 26–31° C. The last filtrate was then passed through a powdered urea column (5 in. × 1/2 in.). After proper elution, 64.7% of methyl linoleate was recovered. Methyl linoleate, thus obtained, was analyzed: I.N. (iodine number), 172.4; isolated *trans*, nil (absorption at 10.32 μ); hydrogen absorption, 1.98 mole/mole. All samples were kept under (dry and oxygen-free) nitrogen and vacuum, below 0° C.

Since we are concerned with the initiation processes only, the extent of oxidation was scrupulously controlled and was not allowed to exceed certain limits. From the previous experiments (10), this limit was set at about 10% oxidation (absorption of 0.1 mole of oxygen per mole which is equivalent to 10% hydroperoxide formation). Consequently, autoxidation of methyl linoleate (50 g) to 10.0% hydroperoxides content was carried out at 0.0° C in 500-ml Erlenmeyer flasks open with loose lids. The quantitative isolation of these hydroperoxides formed at such initial stages and their characterization have been

[1]*Manuscript received January 14, 1959.*
Contribution from the Division of Foods and Nutrition, East Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Tejgaon, Dacca, Pakistan.

considered essential to establish the nature of interactions between oxygen and methyl linoleate containing methylene-interrupted double bonds in a 5-C system. This paper thus deals with the peroxide-forming periods, A and B, and not the final main autoxidation periods, C and D, involving intricate mechanisms with free radicals (10).

Among the methods attempted for isolation of peroxides, the counter-current extraction was found to be the most suitable for keeping the peroxides intact during the isolation procedures. The counter-current extraction between two immiscible solvents was found to be effective in concentrating the oxygenated products. Unluckily, the standard procedures for the counter-current extraction (6) distribute the peroxides over several fractions, and under no conditions is the recovery quantitative. Hence, a scheme of manual counter-current extraction, as represented in Fig. 1, was improvised to separate the

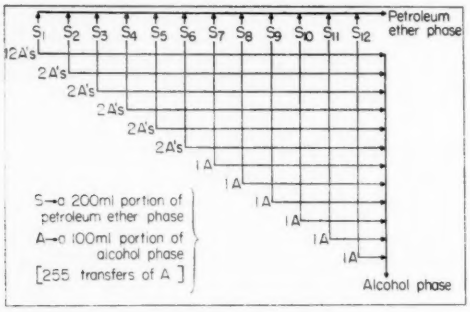


FIG. 1. A scheme of manual counter-current extraction.

oxidized from the unoxidized fraction quantitatively. Two immiscible solvents were first obtained by mixing absolute alcohol (distilled over KOH and zinc dust), petroleum ether (b.p. 30–60° C and free from unsaturable materials (13)), and water in the proportions 5:6:1. The resulting petroleum ether and alcohol phases were allowed to separate and were used as stock solvents. Both the phases were then cooled to 0° C. In Fig. 1, the S rows are 12 separatory funnels, each containing 200 ml of the cold petroleum ether phase. The partially oxidized fatty acid ester was dissolved in the 200 ml of petroleum ether in S_1. Twelve extractions, each with a 100-ml portion (A) of the cold alcohol phase, removed all peroxides from S_1. The subsequent extractions, carried out successively in S_2 through S_{12} by each of these alcohol portions (12 A's, Fig. 1), recovered the majority of peroxides and distributed all unoxidized fatty acid ester together with some peroxides in the petroleum ether phase. These remaining peroxides, in S_2 to S_{12}, inclusive, were extracted by the number of alcohol portions (2 A's and 1 A) indicated at the end of the vertical lines extending from the corresponding petroleum ether phase. This additional extraction of peroxides by fresh solvent is not feasible with the standard counter-current procedures (6). Determination of peroxide values at different steps indicated the efficiency of separation. The peroxide-containing phase of alcohol was kept cooled below 0° C. The precooled oxygen-free nitrogen was bubbled through the above alcohol phase as an added protection. The alcohol phase yielded, in general, 99.0–99.9% of the oxygenated products formed during the autoxidation of fatty acid esters. The unoxidized fraction obtained from the petroleum ether phase contained only 0.0–0.7% of oxygenated products entirely from the unoxidized fatty acid esters, so that the oxygenated products may represent the initial autoxidation process involved. For the convenience of analysis, an aliquot portion

of the oxygenated products was reduced in a 1% alcohol solution by stannous chloride (5 moles/mole of peroxide), agitating with oxygen-free nitrogen.

Methyl linoleate autoxidized at 0° without agitation gave a peroxide concentrate representing 99.9% of the oxygenated products formed during the autoxidation reactions. The corresponding unoxidized fraction isolated was found to be pure methyl linoleate by the following analysis: hydrogen absorption, 1.98 mole/mole; hydroxyl, 0.0; acid value, 0.0; carbonyl value, 0.0; and polymer, 0.0. Elementary analysis: C, 77.47 and H, 11.52. Calculated for methyl linoleate: C, 77.55 and H, 11.56. The polymer contents were measured by the special microtechnique in distillation (15). The peroxide concentrate having the peroxide value of 6115 meq/kg (theory for methyl linoleate monohydroperoxide, 6125 meq/kg was hydrogenated with 2.92 moles H_2 to yield monohydroxystearic acids (m.p. 75.0–77.5° C (3); hydroxyl, 1.04 moles/mole). The preliminary polarographic studies indicated that peroxide concentrate consists of 100.5% of hydroperoxide. The reduced peroxide concentrate gave the following analysis: hydrogen absorption, 1.99 mole/mole; hydroxyl, 1.02 mole/mole; acid value, 0.0; carbonyl value, 0.0 (17); and polymer, 0.0. Elementary analysis: C, 73.41 and H, 10.89. Calculated for monohydroxy methyl linoleate: C, 73.51 and H, 10.97. The infrared analysis of the above samples also indicated production of almost 100% *cis,trans*-conjugated isomers. Two other samples of peroxide concentrates obtained from methyl linoleate autoxidized under identical conditions gave peroxide values of 6100 and 6135 meq/kg respectively. From these data, the initial autoxidation of methyl linoleate (up to extent of 10% used) gives rise to only monomeric hydroperoxides and no other products. The unoxidized fraction has also been isolated as pure methyl linoleate as shown above and by spectrophotometric analyses. Further confirmation of methyl linoleate monomeric hydroperoxides and its specific isomers has been made through the following experiments.

The infrared absorption of the isolated hydroperoxides at 10.55 μ and 10.15 μ for *cis,trans* isomers was compared with that of pure *cis,trans* isomer of methyl linoleate (under the courtesy of the General Mills' Research Laboratory of Minnesota, U.S.A.). Methyl linoleate hydroperoxides were thus proved to consist of only *cis,trans* isomer. Our attempts to separate other isomers, *trans,trans* in particular, from these methyl linoleate hydroperoxides under suitable conditions, ended in failure. About 90 ml of methanol was saturated with urea at 32° C and mixed with cold 10-ml solution of methyl hydroxy linoleates obtained by reduction of linoleate hydroperoxides (2.54 g). It was left to crystallize at 0° C. The crystals were also washed with cold (−10° C) methanol. The washings and mother liquors were combined and reduced in volume to saturation at about 15–20° C under vacuum. The second crop of crystals was collected under the same conditions. The extractions through cold heptane solvent (80–90° C) from cold aqueous solutions gave three fractions, each of which was found to contain *cis,trans* isomers absorbing at 10.15 μ and 10.55 μ. These experiments from the isolation of peroxides were completed in the course of 14 hours on the same day with a well-planned program. The isolated samples sealed under vacuum were kept in dry ice, wherever possible. (N.B. Such hurried activities in these analyses of *cis,trans* isomers were made possible during the tenure of stay by the author in Minneapolis, Minnesota, U.S.A.) A change was definitely found to occur in the *cis,trans* to *trans,trans* ratios of purified autoxidation products (cf. linoleate hydroperoxides and reduced products) after storage for a certain period of time. During the period of experiments, the samples kept under vacuum below 0° C and used occasionally for analyses showed appreciable conversion to *trans,trans* isomers which rose to almost 10% in amounts within the span

of a week. Even storage below $-50°$ under vacuum did not stop such conversion. The *trans,trans* isomer (more than 25%) was obtained in all samples in a period of 6–9 months. Hence, it is quite apparent that the experimental conditions, unless scrupulously controlled, may change the true picture of autoxidation products consisting of only *cis,trans* isomer at the initial stages. The survey of literature (3, 5) indicates that all workers who studied kinetics or proposed theories did not consider these initial stages of autoxidation as important and never maintained conditions for keeping the peroxides intact in structure. Consequently, they dealt with only main autoxidation reactions involving decomposition of these peroxides and the resultant free radicals. Our point is related to only where and how autoxidation begins and, hence, much importance is attributed to the initial products unchanged and their energy of formation (10).

Characterization of Methyl Hydroxy Linoleates

Methyl hydroxy linoleates obtained by reduction of hydroperoxides from initial autoxidation of methyl linoleate have been previously shown to be *cis,trans*-conjugated isomers only through infrared analyses. Such products (250 mg) were quantitatively hydrogenated by Adam's platinum catalyst (1) to absorb 1.97 moles H_2 per mole. Methyl hydroxy stearates were subjected to resolution by column chromatography. Alumina was washed with 2 *N* HCl, then with water until neutral, and finally with ethanol. On activation at 150° C for 6 hours, the alumina was poured into a tube (1.0 cm × 110.0 cm) filled with petroleum ether (freed of the unsaturable materials and b.p. 80–90° C). Elution was carried on through petroleum ether containing 0–14% chloroform. Suitable fractions were collected and, through selections, the representative samples were made up. Methyl hydroxy stearates were thus resolved into two fractions (yield, 98.1% of that used) of almost equal weight. These had melting points: first 52.8–51.1° C (found: C, 72.4; H, 12.8; $C_{19}H_{33}O_3$ requires: C, 72.6; H, 12.1) and second 50.8–51.2° C (found: C, 73.2; H, 12.4). They were identified as methyl-, 13-, and 9-hydroxy stearates as done by Bergstrom (4).

Monohydroxystearic acids (40–50 mg) from methyl esters were added to 25% sulphuric acid (3.5 ml) containing 8% chromic acid. Steam was passed through the mixture for about 55 minutes with volume kept constant by additional controlled heating. The distillate was neutralized with alkali. After some concentration under vacuum, the acids were regenerated by sodium bisulphate and extracted with *n*-butanol. The scheme of partition paper chromatography (12) was utilized to analyze these acids; three well-defined intense spots were obtained, two of which had R_f values identical with those of *n*-pentanoic and *n*-hexanoic acids, and the third spot was in a some doubtful position of acids greater than C_8 in chain length. The other faint spots were neglected. For further confirmation, the method of Michael and Schweppe (14) was employed for detecting acids of chain length C_5–C_{18}. The butanol extracts were esterified by means of diazomethane (2) and the corresponding hydroxamic acids were prepared, and the acids *n*-pentanoic, *n*-hexanoic, *n*-nonanoic, and *n*-decanoic were identified. These four acids: C_5, C_6, C_9, and C_{10} can only result from the 9- and 13-hydroxystearic acids, confirming our results in the foregoing section.

Some Concepts on Mechanisms of Autoxidation of Methyl Linoleate

From the above findings, it can be asserted that only two *cis,trans*-conjugated isomers of 9- and 13-linoleate hydroperoxides are formed during autoxidation reactions. No 11-hydroperoxide has been detected in the products. Hence, the formation of 11-peroxide radical ($R^{11}O_2^{\cdot}$) and transfer of O_2 to 9- and 13-position seems to be absurd from the modern concepts (cf. transfer of atoms) of electronic interpretation (8, 16). Furthermore,

the initiation of reaction through free radicals on the 5-C systems of linoleate does not eliminate the formation of 11-isomer. This has been confirmed by the excellent work on free radicals attacking such 5-C systems by Harrison and Wheeler (7). The question of energy to start off the first radical during the interactions of oxygen and linoleate can not be easily thrown off (10, 11).

It is, thus, quite apparent that complex formation already proposed for simple systems (10) may be applied to 5-C systems of methyl linoleate with some modifications. The concepts of non-localization and spread of π electrons (8) have made it possible to present the processes of activation and polarization of 5-C systems and oxygen molecules in the steps I–III of Fig. 2. The first stages of activated complex formation (IV) may involve

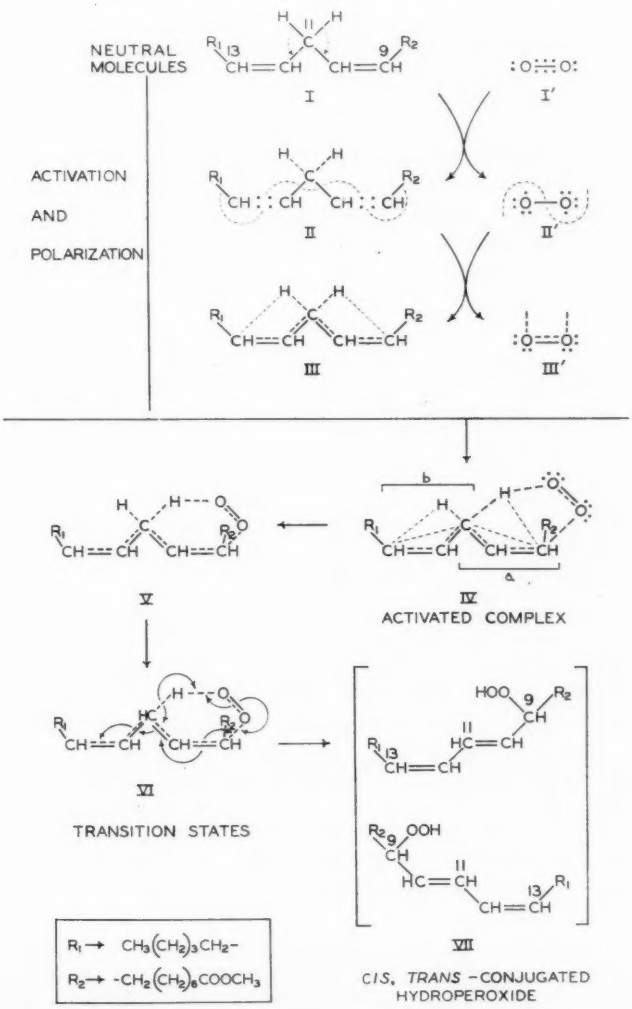


FIG. 2. Mechanism of autoxidation of methyl linoleate.

either *a* or *b* segment of the 5-C system. The activated complex through *a* (IV) gets its π electrons spread over the whole sphere of reactions including oxygen molecule (V) and then initiates shift of electrons as in VI, Fig. 2, only to give rise to the isomer, 9-hydroperoxide. Similarly, the activated complex through segment *b* (IV) will form 13-isomer of hydroperoxide. That the *cis* to *trans* conversion occurs only in one affected double bond next to hydroperoxide group, is evident from VI–VII, Fig. 2, showing spatial possibilities for 9-isomer. The foregoing mechanisms proposed for methylene-interrupted double bonds (5-C system) of methyl linoleate may account for the clear-cut formation of two conjugated isomers: 9- and 13-*cis*,*trans*-hydroperoxides that have been found as the reaction products of oxygen and methyl linoleate at the initial stages of autoxidation.

It may be noted here that it is of particular interest to consider the above mechanisms as the missing link between neutral methyl linoleate and the formation of its hydroperoxides without decomposition. After peroxide formation, many complicated processes including free radicals may be involved for the main autoxidation reactions which have been studied by various workers, kinetically or otherwise (3, 5). Hence, the proposed mechanisms for the initial reactions do not replace the ideas of free radicals, but these processes only supplement free radical mechanisms that play roles quite later and, consequently, demand careful kinetic studies from a different angle of approach under the conditions prescribed in this paper. Our future plans include such kinetic investigations. Moreover, the mode of complex formation, ahead of free radical reactions, is not new and has been found in many other reaction processes (18). Further details will be published later.

REFERENCES

1. ADAMS, R., VOORHEES, V., and SHRINER, R. L. Org. Syntheses, Coll. Vol. **I**, 463 (1948).
2. ARNDT, F. Org. Syntheses, Coll. Vol. **II**, 165, 461 (1943).
3. BATEMAN, L. Quart. Revs. **8**, 147 (1954).
4. BERGSTROM, S. Arkiv. Kemi, Mineral. Geol. A, **21**, 14 (1945); Nature, **156**, 717 (1945).
5. BOLLAND, J. L. and GEE, G. Trans. Faraday Soc. **42**, 239 (1946).
6. CRAIG, L. C. and CRAIG, D. *In* Technique of organic chemistry. Vol. III. *Edited by* A. Weissberger. Interscience Publishers, Inc., New York. 1950. p. 259.
7. HARRISON, S. and WHEELER, D. H. J. Am. Chem. Soc. **76**, 2379 (1954).
8. HERMANS, P. H. Introduction to theoretical organic chemistry. *Edited and translated by* R. E. Reeves. Elsevier Press Inc., New York. 1954. pp. 71–75.
9. KHAN, N. A. Biochim. et Biophys. Acta, **16**, 159 (1955).
10. KHAN, N. A. Can. J. Chem. **32**, 1149 (1954).
11. KHAN, N. A. J. Am. Oil Chemists' Soc. **30**, 273 (1953).
12. LINDQUIST, B. and STORGÅRDS, T. Acta Chem. Scand. **7**, 87 (1953).
13. MELVILLE, H. W. and COOPER, H. R. J. Chem. Soc. 1988 (1951).
14. MICHAEL, F. and SCHWEPPE, H. Mikrochim. Acta, 53 (1954).
15. PASCHKE, R. F., KERNS, J. R., and WHEELER, D. H. J. Am. Oil Chemists' Soc. **31**, 5 (1954).
16. REMICK, A. E. Electronic interpretations of organic chemistry. 2nd ed. John Wiley & Sons, Inc., New York. 1949. p. 112.
17. SIGGIA, S. Quantitative organic analysis via functional groups. John Wiley & Sons, Inc., New York. 1949.
18. URI, N. J. Am. Chem. Soc. **74**, 5808 (1952). BOOZER, C. E. and HAMMOND, G. S. J. Am. Chem. Soc. **76**, 3861 (1954). MAYO, F. R. J. Am. Chem. Soc. **75**, 6133 (1953).

A COMPARISON OF SLATER'S THEORY OF UNIMOLECULAR REACTIONS WITH EXPERIMENTAL DATA[1]

EVERETT THIELE AND DAVID J. WILSON

ABSTRACT

Slater's theory of unimolecular reactions is tested by comparing theoretical and experimental values of the pressure at which the rate constant has fallen off from the high-pressure limit by 5%. A simplified method is used for calculating lower bounds to the theoretical value of this pressure. Agreement with experimental data on cyclobutane, cyclobutene, and nitrogen pentoxide is rather poor; experimental values are lower than the calculated lower bounds.

Slater has set forth a detailed theory of unimolecular gas reactions which is based on a model of a vibrating molecule with a quadratic potential function (1, 2, 3). The molecule reacts when one particular internal co-ordinate (the reaction co-ordinate) attains a critical value. Slater derives an expression for the value of the rate constant at any pressure (k) divided by the value of the rate constant at infinite pressure (k_∞). His expression is $k/k_\infty = I_n(\theta)$, where the right-hand side is an integral depending on n, the number of normal co-ordinates contributing to the reaction co-ordinate, and on θ, which is proportional to pressure. Slater has evaluated this integral for $n = 3, 5, \ldots 13$ at increments of one of $\log_{10}\theta$. For our purposes it is sufficient to determine the value of θ for which $k/k_\infty = 0.95$, from which we shall determine the pressure (P_5) at which the reaction rate has dropped off 5% from its value at the high-pressure limit. Slater has also tabulated these values (3).

The exact application of Slater's theory to a reaction requires a detailed vibrational analysis, a knowledge of the experimental activation energy and of the reaction co-ordinate, and values for the collision diameters of the reactant and any added inert gases. In general, the larger the value of n, the lower is the predicted value of P_5. One upper bound for n is obviously the total number of normal modes of vibration possessed by the reactant molecule. In a molecule possessing symmetry, however, n may be considerably smaller than this if the reaction co-ordinate belongs to one of the symmetry species of the molecule. Even when this is not the case, an upper bound for n can often be determined by considering the symmetry of the less symmetrical molecule resulting when the molecule is distorted by giving the reaction co-ordinate a large value.

For Slater's quantity ν (for definitions of Slater's parameters the reader is referred to ref. 3) one can substitute the high-pressure limit of the pre-exponential factor. This leaves undetermined only the value of Slater's $\Pi_i\mu_i$. A maximum value of this quantity makes P/θ, and also P_5, a minimum. This maximum value is given by Slater (3) as $n^{-\frac{1}{2}n}$, and is obtained by assigning to all the μ_i an equal value. Physically this means that all pertinent normal modes contribute equally to the reaction co-ordinate. Slater has also defined a "representative spread" of the μ_i in such a way that they form a geometric progression with $\mu_1 = 5\mu_n$. P_5 has been calculated for both cases (and is defined as $P_{5\,\mathrm{min}}$ and $P_{5\,\mathrm{rep}}$, respectively) for three unimolecular reactions: the isomerization of cyclobutene, the decomposition of cyclobutane, and the decomposition of nitrogen pentoxide.

Cooper and Walters (4) concluded that the isomerization of cyclobutene to 1,3-butadiene proceeds unimolecularly without the formation of free radicals. If one assumes

[1]*Manuscript received January 29, 1959.*
Contribution from Department of Chemistry, University of Rochester, Rochester 20, New York.

C_{2v} symmetry for the molecule, the reaction co-ordinate is probably of symmetry species A_1. (One expects the reaction co-ordinate to be a simultaneous lengthening of the CH_2—CH_2 and CH=CH bonds and shortening of the CH_2—CH bonds; hydrogen migration seems unlikely and would lead to a too-large pre-exponential factor.) There are eight normal co-ordinates belonging to this species; therefore no more than eight normal vibrations can contribute to the reaction co-ordinate.

The decomposition of cyclobutane has been investigated by several authors (5, 6 *a*) and is thought to be elementary unimolecular. A possible reaction co-ordinate is the simultaneous expansion of two opposite C—C bonds. Although Slater's theory does not apply directly to such a case, we can hypothesize that the sum of these two bond lengths must reach a critical value in order for the reaction to take place. If one considers cyclobutane in such a stretched position prior to reaction, and assumes D_{2h} symmetry, the reaction co-ordinate is a member of the A_g symmetry species, which contains five normal modes.

Alternatively, the stretching of only one C—C bond may be necessary for reaction, in which case the distorted molecule prior to reaction has C_{2v} symmetry. The reaction co-ordinate is of symmetry species A_1, and thus no more than nine normal modes can contribute to it.

An argument against the participation of hydrogen migration to the reaction co-ordinate of cyclobutane is the result of recent unpublished work by Srinivasan and Kellner (both at the University of Rochester), who found that the thermal decomposition of 1,1,2,2-tetradeuterocyclobutane produced essentially no ethylene species of odd molecular weight. We are grateful to these workers for permission to quote their results. (It might be remarked in passing that the work of Rabinovitch and co-workers on dideuterocyclopropane (6 *b*) casts some doubt on the use of a hydrogen migration reaction co-ordinate in the analysis of data on the isomerization of cyclopropane.)

The rate-controlling step in the decomposition of nitrogen pentoxide in the presence of nitric oxide has been found to be unimolecular (7, 8, 9, 10). The reaction co-ordinate might be taken to be the antisymmetric migration of the central oxygen atom away from one nitrogen and toward the other. If, for purposes of computation, the molecule is fixed in a planar configuration, it possesses C_{2v} symmetry. The reaction co-ordinate belongs to symmetry species B_1, and so can be a combination of no more than five normal modes.

Alternatively, the reaction co-ordinate might be the stretching of one N—O bond. For a planar configuration, the distorted molecule possesses C_s symmetry, and 11 normal modes can contribute to the reaction co-ordinate.

Values of $P_{5\,\min}$ and $P_{5\,\max}$ can then be computed for these reactions by means of Slater's tables. (θ_5 for $n = 8$ was calculated by interpolation in Slater's table.) A collision diameter of five Angstroms was used in all calculations.

In two cases there exists a duplication of equivalent reaction co-ordinates within the molecule. Slater has shown that the effect of such a duplication is approximately equal to a multiplication of the over-all rate constant by the degree of duplication (11). Hence no change in the pressure at which $k/k_\infty = 0.95$ should be expected because of this duplication. The results of these calculations and the parameters used are listed in Table I.

It might be noted that, assuming C—C bond stretching is the reaction co-ordinate, Slater's theory is unable to account for the high pre-exponential factor found for cyclobutane. Other examples of "high" pre-exponential factors are known. It has also been

TABLE I

	Experimental data				Theoretical results					
	ν ($\times 10^{13}$ sec^{-1})	E_0 (kcal/mol)	T (° K)	$P_{\frac{1}{2}}$ (mm)		n	$f_{n\,\max}$	$P_{\frac{1}{2}\,\min}$ (mm)	$f_{n\,\mathrm{rep}}$	$P_{\frac{1}{2}\,\mathrm{rep}}$ (mm)
Cyclobutene	1.2	32.5	422	10		8	20	1100	2.7	8500
Cyclobutane	400	62.5	722	50	Case 1	5	5.65	1.0×10^{7}	1.3	4.4×10^{7}
					Case 2	9	30	1.2×10^{5}	3.3	1.1×10^{6}
Nitrogen pentoxide	50	21	300	1×10^{4}	Case 1	5	5.65	1.7×10^{6}	1.3	7.5×10^{6}
					Case 2	11	70	6.3×10^{3}	5.3	8.3×10^{4}
					Interpolation was used for $n = 8$					

found experimentally that deactivation does not necessarily occur on every collision of an activated molecule with a foreign gas molecule (12, 13); hence the estimate of five Angstroms for the effective collision diameter may well be too high. This would introduce even larger discrepancies between the results of theory and experiment given in Table I.

One concludes that Slater's theory, although certainly the most detailed of the available theories of unimolecular reactions, may require some modifications—perhaps the inclusion of fairly substantial anharmonic interactions between the normal modes, and perhaps the use of a different type of collisional activation mechanism.

REFERENCES

1. SLATER, N. B. Proc. Cambridge Phil. Soc. **35**, 56 (1939).
2. SLATER, N. B. Proc. Roy. Soc. A, **194**, 112 (1948).
3. SLATER, N. B. Phil. Trans. Roy. Soc. London, A, **246**, 57 (1953).
4. COOPER, W. and WALTERS, W. D. J. Am. Chem. Soc. **80**, 4220 (1958).
5. GENAUX, C. T., KERN, F., and WALTERS, W. D. J. Am. Chem. Soc. **75**, 6196 (1953).
6. (*a*) PRITCHARD, H. O., SOWDEN, R. C., and TROTMAN-TICKENSON, A. F. Proc. Roy. Soc. A, **218**, 416 (1953).
   (*b*) RABINOVITCH, B. S., SCHLAG, E. W., and WIBERG, K. B. J. Chem. Phys. **28**, 504 (1958).
7. SMITH, J. H. and DANIELS, F. J. Am. Chem. Soc. **69**, 1735 (1947).
8. MILLS, R. L. and JOHNSTON, H. S. J. Am. Chem. Soc. **73**, 938 (1951).
9. JOHNSTON, H. S. and PERRINE, R. L. J. Am. Chem. Soc. **73**, 4782 (1951).
10. HISATSUNE, I. C., CRAWFORD, B., and OGG, R. A., JR. J. Am. Chem. Soc. **79**, 4648 (1957).
11. SLATER, N. B. Proc. Roy. Soc. A, **218**, 224 (1953).
12. CORDES, H. F. and JOHNSTON, H. S. J. Am. Chem. Soc. **76**, 4264 (1954).
13. WILSON, D. J. and JOHNSTON, H. S. J. Am. Chem. Soc. **75**, 5763 (1953).

THE WAX OF THE LEAVES OF PICEA PUNGENS (COLORADO SPRUCE)[1]

E. VON RUDLOFF

ABSTRACT

A chloroform-soluble, neutral wax was isolated from the leaves of Colorado spruce, collected during fall and winter months. The wax was found to consist of sabinic, 14-hydroxytetradecanoic, and juniperic acids, with only minor amounts of non-saponifiables and *n*-fatty acids. 14-Hydroxytetradecanoic acid has not been found before in naturally occurring waxes. A cyclic structure rather than the linear etholides proposed for other conifer waxes is in accord with the analytical data. In the spring and summer months the wax was accompanied by an acidic fraction.

A wax isolated from the leaves of Colorado spruce (*Picea pungens* Engelm.) has been found to differ from the waxes of other conifer leaves (1–7). Leaves harvested in the fall or winter yielded a virtually neutral wax which contained only minor amounts of non-saponifiable material. One of its main constituents is 14-hydroxytetradecanoic acid, which has apparently not been found in natural waxes before. Samples obtained during the spring or summer months, however, had much higher acid values, indicating that seasonal changes occur. Yields of the wax and analytical data are shown in Table I.

TABLE I

Yield and analytical data of waxes

Date of harvest		% Yield (based on dry weight)	Melting point, °C	Acid value*	Saponification value*	%C	%H	Molecular weight	% Non-saponifiables
Sept./Oct.	1957	0.2–0.25	71–74	0–2	248†	72.92	11.09	860–900	3–4
Dec.	1957	0.25	70–74	0–2	252	—	—	—	—
March/April	1958	0.35	70–73.5	56	184	72.47	11.47	770–800	2–3
May	1958	0.35	69–74	54	—	—	—	—	—
July	1958	0.25	69–74	35	—	—	—	—	—
November	1958	0.25	70–73	5–6	225	74.43	11.77	900–920	1–2
Neutral portion									
March/April	1958	—	70–75	3–5	210	74.36	11.81	920–960	1–2

*Expressed in mg KOH per g wax.
†Saponification value after acetylation: 246.

The neutral wax, after purification, was saponified and the mixture of acids obtained on acidification was fractionated by countercurrent distribution (8). Three distinct fractions, comprising about 90% of the mixture, were obtained after applying 300 or more transfers, and were found to contain sabinic, 14-hydroxytetradecanoic, and juniperic acids, respectively (see Table II). The remaining 10% of the acid mixture was distributed broadly without forming a distinct peak. This was due in part to emulsion formation in tubes containing the less polar material (i.e. normal fatty acids). When the methyl esters were similarly fractionated no emulsification was encountered and the partition of the hydroxy compounds was more favorable, but again no distinct peak was obtained for the less polar compounds. Analysis by vapor-phase chromatography showed that normal fatty acids from lauric to stearic acids were present (see Table II). A substantial seasonal variation was found in the composition of the ω-hydroxy and normal fatty acid

[1]*Manuscript received February 16, 1959.*
Contribution from the National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Saskatchewan.
Issued as N.R.C. No. 5168 and as Paper No. 259 on the Uses of Plant Products.

mixtures. When the acidic wax isolated in the spring was chromatographed on a silicic acid – celite column a high proportion was recovered as a neutral wax having a composition similar to that isolated in the fall. The small amount (1–4%) of non-saponifiable material isolated had an infrared spectrum which resembled that of *n*-octadecanol; however vapor-phase chromatography showed it to be a mixture of at least four compounds.

TABLE II

Composition of the acidic portion of waxes

Date of harvest		ω-Hydroxy acids			*n*-Fatty acids			
		C_{12}	C_{14}	C_{16}	C_{12}	C_{14}	C_{16}	C_{18}
Sept./Oct.	1957*	22	40	28	(together about 10%)			
Sept./Oct.	1957†	18	45	26	Trace	2	4	5
March/April crude	1958†	13.5	36.5	12.5	7.5	5	10	14.5
March/April neutral	1958†	22.5	44	20.5	2.5	1.5	3	6
November	1958†	17	41	20	Trace	2	5	3

*Countercurrent distribution.
†Vapor-phase chromatography.

The neutrality of the wax, the small percentage of non-saponifiable material present, and the molecular weight of 850 to 950 suggest that the pure wax is composed of about four molecules of the three ω-hydroxy acids, esterified with each other in form of a cyclic polymer. Bougault and Bourdier (1) concluded that the waxes which they isolated consisted of similar esters but in the form of linear polymers, which they named etholides. Such an etholide (m.p. 87.5–88° C) has been prepared from juniperic acid by heat condensation (9). Kariyone *et al.* (5) deduced from their data that such etholides are composed of three to five molecules of juniperic and sabinic acids. A linear polymer would require one free hydroxyl and one free carboxyl group for each molecule. Assuming an average composition corresponding to a linear tetramer of 14-hydroxytetradecanoic acid, the calculated acid value, neutral equivalent, saponification value, and saponification equivalent after acetylation would be 60.7, 923.4, 230.8, and 965.4 respectively. These data do not correspond with those found for either the neutral or acidic wax from Colorado spruce (Table I). Infrared spectra of the neutral wax showed no free hydroxyl groups, and only a small peak for the acidic wax. It is suggested, therefore, that the neutral wax is composed mainly of a cyclic polymer of the ω-hydroxy acids and that the seasonal changes in acidity are caused, in part at least, by ring opening or closure.

Bougault and Cattelain (10) and Tong (7) have suggested that juniperic acid could be used for the production of compounds having musk odor, but the former authors thought the yields too small to be of practical value. Since 14-hydroxytetradecanoic acid forms a 1,14-tetradecanolide having musk odor (11), the same suggestion would apply to the wax from Colorado spruce leaves.

EXPERIMENTAL

Melting points were measured with a Leitz hot stage microscope. Vapor-phase chromatography was carried out with a Beckman GC-2 gas chromatograph using a 2-foot long, ¼-inch diameter column of washed silicone on C-22 firebrick (12) and helium as carrier gas. A modified E-C Apparatus Co. Fractionator, Model EC 510, was used in the

countercurrent distribution experiments (13). Molecular weights were determined by the Rast and isothermal distillation methods (14). Acid values (neutralization equivalents) and saponification equivalents were determined in the usual way, using 90% ethanol as solvent. Because of the low solubility of the waxes, a small amount of chloroform was added and titrations were carried out at 50–60° C. Acetylations were carried out with acetyl chloride and pyridine or dimethylaniline.

Isolation of the Wax

Leaves harvested in the fall of 1957 and spring of 1958 were treated as follows: fresh leaves, 200 g (dry weight approx. 90 g), were macerated in hot 40–60% aqueous alcohol, 1500 ml, with the aid of a 1-gal Waring blendor. The mixture was filtered and the residual pulp extracted similarly with hot benzene – ethanol (2:1 v/v). Both solutions, on reduction to a small volume and cooling, precipitated waxy material which was filtered off. The crude wax was found to be soluble in cold chloroform, hot ethanol, hot acetone, hot benzene, and hot carbon tetrachloride, and was insoluble in water, ether, and petrol.

In later work, the cuticle wax was isolated directly by steeping the fresh needles, 100 g, in chloroform, 500 ml. After it had been left standing for 2 to 3 hours the mixture was filtered and evaporated *in vacuo* (water pump) in a 1-liter flask. The residual material, 1.5 g, formed a thin film along the walls of the flask and was triturated with ether to remove ether-soluble impurities, 0.9 g.

The yields of crude wax at different times of the year are shown in Table I.

Purification of the Wax

The crude wax, 1 g, was dissolved in hot 75% aqueous alcohol (100 ml), and to the solution at 50–60° C was added an equal volume of petrol (b.p. 60–70° C). The mixture was stirred carefully and allowed to cool to room temperature, and the precipitated wax was filtered off. If the wax still had a light green color, the procedure was repeated. The wax was then recrystallized from acetone two or three times to give a constant melting product. If the wax was then further recrystallized from ethanol, ethanol–acetone, or petrol, the melting-point range did not decrease. The constants and analytical data for waxes isolated at various times are shown in Table I.

Separation into Components

The neutral wax (isolated in the fall of 1957, neutral equivalent 267), 1.000 g, was saponified by heating under reflux in 80% aqueous ethanol, 50 ml, containing potassium hydroxide, 1.0 g, for 6 to 8 hours. The non-saponifiable portion was recovered quantitatively by extracting the cold, diluted ethanolic solution three times with an equal volume of petrol (b.p. 60–70° C). The petrol extract was dried over anhydrous sodium sulphate, filtered, and evaporated to dryness. The residue, 0.010 g, had m.p. 51–70° C and an infrared spectrum resembling that of *n*-octadecanol, though not as well defined. Vapor-phase chromatography showed the presence of at least four compounds.

The ethanolic soap solution was evaporated to a small volume, acidified with 10% sulphuric acid solution, and quantitatively extracted with ether. The dried, filtered ethereal solution was evaporated to dryness to yield the free acids, 1.037 g (97.2% of theory, assuming the wax to be composed of etholides only). In another experiment the yield of non-saponifiables was 0.032 g and that of acids 1.040 g (97.6%).

The mixture of acids, 2.0 g, was fractionated by countercurrent distribution, using 60% aqueous ethanol – petrol (b.p. 60–70° C) as solvent system. When 200 transfers were applied three major peaks were obtained (weight analysis) which were not well

separated. Applying 400 transfers (withdrawal technique) a better than 90% separation of each component was obtained. Emulsification was troublesome in the most advanced tubes, and a better fractionation was obtained when the methyl esters were used (see below). The amount of each component was calculated and the material at and adjacent to the maxima was combined, evaporated, and recrystallized from acetone. Fraction I (tubes 0–18), 0.42 g (21%), m.p. 82–83.5° C, corresponding to sabinic acid, m.p. 84° C, 84–85° C (1, 9). Calculated for $C_{12}H_{24}O_3$: C, 66.63%; H, 11.18%; neutral equivalent, 216.31. Found: C, 66.91%; H, 11.18%; neutral equivalent, 218.2. The methyl ester was prepared with diazomethane, giving a compound m.p. 31–34° C after three crystallizations from petrol and methanol–water; reported m.p. 34–34.5° C (9). Calculated for $C_{13}H_{26}O_3$: C, 67.78%; H, 11.38%; saponification equivalent, 230.34. Found: C, 68.01%; H, 11.45%; saponification equivalent, 238.3. Acetyl derivative m.p. 42–43.5° C after three crystallizations from petrol (b.p. 60–70° C); reported m.p. 45° C (9).

Fraction II (tubes 19–40), 0.80 g (40%), m.p. 89.5–91.0° C; reported for 14-hydroxytetradecanoic acid, m.p. 91.0–91.5° C (9). Calculated for $C_{14}H_{28}O_3$: C, 68.81%; H, 11.55%; neutral equivalent, 244.36. Found: C, 68.54%; H, 11.35%; neutral equivalent, 246.36. Methyl ester: m.p. 45.5–46.0° C; reported m.p. 47° C (9). Calculated for $C_{15}H_{30}O_3$: C, 69.72%; H, 11.70%; saponification equivalent, 258.39. Found: C, 69.93%; H, 11.79%; saponification equivalent, 259.2. Acetyl derivative: m.p. 53.5–54.5° C; reported m.p. 54–54.5° C (9).

Fraction III (tubes 41–66), 0.56 g (28%), m.p. 94.5–95.5° C; reported for juniperic acid m.p. 95° C (1, 9). Calculated for $C_{16}H_{32}O_3$: C, 70.54%; H, 11.84%; neutral equivalent, 272.42. Found: C, 70.26%; H, 11.60%; neutral equivalent, 274.5. Methyl ester: m.p. 54.5–55.0° C; reported m.p. 55–55.5° C (1, 9). Calculated for $C_{17}H_{34}O_3$: C, 71.28%; H, 11.96%; saponification equivalent, 286.44. Found: C, 71.17%; H, 11.62%; saponification equivalent, 283.5. Acetyl derivative: m.p. 62–64° C; reported m.p. 63° C (9).

The material in tubes 67 and higher, 0.15 g (7.5%), formed no distinct peak. It was combined, esterified, and analyzed by vapor-phase chromatography. Peaks corresponding to lauric (approximately 5%), myristic (20%), palmitic (35%), and stearic (40%) acids were obtained.

The mixture of acids was also fractionated by countercurrent distribution in form of their methyl esters. A satisfactory separation into three hydroxy esters was obtained with 200 transfers using 75% aqueous ethanol – petrol (b.p. 60–70° C) as solvent system. The partition ratios for the C_{12}, C_{14}, and C_{16} ω-hydroxy esters were 0.18, 0.33, and 0.48, respectively, and the relative amounts of each component were practically the same as those found for the free acids.

Chromatography of Acidic Wax

The acidic wax (isolated in the spring of 1958, acid value 56), 1.000 g, was chromatographed on a column, 2.0 cm diameter, of silicic acid – celite (2:1), 50 g, using chloroform as eluent. Fractions of 25 ml were collected. Fractions 5 to 11 yielded 0.93 g of practically neutral wax, m.p. 70–75° C. Found: C, 74.36%; H, 11.81%; acid value 3–5; saponification equivalent, 267; molecular weight, 940–970. Saponification equivalent after acetylation, 272. A small aliquot was saponified and the free acids converted to their methyl esters with diazomethane. The percentage composition of the mixture of methyl esters is shown in Table II.

Vapor-phase Chromatography

All material investigated was injected as concentrated ethereal solutions of the methyl

esters, or, in the case of the non-saponifiables, as dilute ethanolic solutions of the free alcohols. The methyl esters were analyzed at 220° C and 30 p.s.i. pressure (flowrate = 82.55 cc per minute, soap bubble counter). The time of emergence, measured on the chart paper as the distance from the start (solvent peak) to the maximum of each peak, was 1.8, 3.5, 7.0, and 13.9 (±0.2) cm for methyl laurate, myristate, palmitate, and stearate and 5.4, 9.7, and 20.1 (±0.2) cm for the methyl esters of the C_{12}, C_{14}, and C_{16} ω-hydroxy acids, respectively. The areas under the curves were obtained by the triangulation method and the percentage of each component was calculated from its area as compared with the sum of the peak areas. The error on runs with mixture of known amounts of normal fatty acid esters was ±1%. When the non-saponifiable material was analyzed on the same column and at the same temperature, four peaks were recorded.

ACKNOWLEDGMENTS

The author is grateful to Mr. M. Mazurek for carrying out the microanalyses and to Mr. M. Granat for technical assistance and recording the infrared spectra.

REFERENCES

1. Bougault, J. and Bourdier, L. Compt. rend. **147**, 1311 (1908); J. pharm. chim. **29** (6), 561 (1909); **30**, 10 (1909); **3** (7), 101 (1911).
2. Kaufmann, H. P. and Friedebach, M. Chem. Ber. **55**, 1508 (1922).
3. Sakurai, J. J. Pharm. Soc. Japan, **51**, 34 (1931); **53**, 579 (1933).
4. Fujita, A. and Yoshikawa, T. J. Pharm. Soc. Japan, **71**, 913 (1951).
5. Kariyone, T., Watanabe, H., and Kadowaki, H. J. Pharm. Soc. Japan, **72**, 5, 7, 10, 11 (1952).
6. Kariyone, T. and Isoi, K. J. Pharm. Soc. Japan, **74**, 1312 (1954); **75**, 316 (1955); **76**, 473 (1956).
7. Tong, S. T. Soap, Perfumery & Cosmetics, **27**, 58 (1954).
8. Horn, D. N. S., Hougen, F. W., Rudloff, E. von, and Sutton, D. A. J. Chem. Soc. 177 (1954).
9. Chuit, P. and Hausser, J. Helv. Chim. Acta, **12**, 463 (1929).
10. Bougault, J. and Cattelain, E. Compt. rend. **186**, 1746 (1928).
11. Crabalona, L. L. Brit. Pat. No. 655,428 of July 18, 1951. cf. Chem. Abstr. **46**, 8148ᵃ (1952).
12. Craig, B. M. and Murty, N. L. Can. J. Chem. **36**, 1297 (1958).
13. Raymond, S. Anal. Chem. **30**, 1214 (1958).
14. Childs, C. E. Anal. Chem. **26**, 1963 (1954).

THE BIOGENESIS OF ALKALOIDS

XXI. THE BIOGENESIS OF (−)-HOMOSTACHYDRINE AND THE OCCURRENCE OF TRIGONELLINE IN ALFALFA[1]

A. V. ROBERTSON[2] AND LÉO MARION

ABSTRACT

In an attempt to find the precursor of the piperidine ring in homostachydrine, young alfalfa shoots were fed (*a*) lysine-2-^{14}C, (*b*) lysine-2-^{14}C with pyridoxine, (*c*) lysine-2-^{14}C with pyridoxine and folic acid, and finally (*d*) lysine-2-^{14}C with pyridoxine, folic acid, and methionine. In all cases the homostachydrine isolated from the harvested plants showed no radioactivity. It is shown that homostachydrine is not synthesized at the stage of growth at which the experiments were carried out. Alfalfa has been found to contain a hitherto unreported alkaloid shown to be trigonelline by comparison with an authentic sample. Both homostachydrine and trigonelline are found in the seeds, and are not actively synthesized in the plant until seed formation. Asparagine has also been isolated in relatively high yield.

In the course of experiments on the biosynthesis of stachydrine in alfalfa (*Medicago sativa* L. Grimm) Wiehler and Marion isolated a new alkaloid which they called (−)-homostachydrine (1). The compound was identified as the laevorotatory methyl betaine of pipecolic acid, and it has since been shown to belong to the L-series of amino acids by correlation with L-(−)-pipecolic acid (2, 3).

It has been suggested (1) that homostachydrine is formed in the plant by ring closure of lysine to pipecolic acid followed by the methylation to the betaine. Lysine is known to be a precursor of pipecolic acid in *Phaseolus vulgaris* (4, 5), *Neurospora* (6), and the intact rat (7), and the intermediates of the process have been studied with the aid of enzyme preparations from rat tissue, *Neurospora*, *Pisum sativum*, and *Phaseolus radiatus* (8, 9). The presence of pipecolic acid in alfalfa has yet to be demonstrated but in view of its occurrence in other legumes (10, 11, 12) it would not be surprising if it were found as a nonprotein nitrogen constituent of alfalfa.

We have attempted to provide evidence for the biosynthetic pathway proposed for homostachydrine by feeding lysine-2-^{14}C to 2-week-old alfalfa. A week later the plants were harvested and extracted, but the homostachydrine isolated was radioinactive. In previous experiments on the biosynthesis of stachydrine it was found that ornithine-2-^{14}C was not converted to proline in alfalfa unless pyridoxine was also administered, but even then the stachydrine did not become radioactive (13). In case similar conditions prevailed for the ring closure of lysine, an experiment was performed in which lysine-2-^{14}C and pyridoxine were fed to 2-week-old alfalfa, but again the crystalline alkaloid fraction isolated from the plants a week later was not radioactive. Following the ideas of Wiehler and Marion (14) on stachydrine biosynthesis, folic acid was fed together with lysine-2-^{14}C and pyridoxine in an attempt to stimulate the biological methylation of pipecolic acid, but no radioactivity was incorporated in the homostachydrine subsequently isolated. In a final experiment to influence the biosynthesis of homostachydrine along the proposed route by enhancing the probability of methylation, lysine-2-^{14}C was fed in conjunction with pyridoxine, folic acid, and methionine, but again the alkaloid remained inactive.

These negative results might be interpreted to mean either that lysine is not a pre-

[1]*Manuscript received March 3, 1959.*
Contribution from the Division of Pure Chemistry, National Research Council, Ottawa, Canada.
Issued as N.R.C. No. 5179.
[2]*National Research Council of Canada Postdoctorate Fellow (1957–59).*

cursor of homostachydrine, or that the synthesis of the alkaloid takes place at a later stage of development of the plant, in which case the homostachydrine isolated would have been material stored in the seeds. The second of these interpretations is supported by a result described by Wiehler and Marion (14), who found that after feeding methionine-Me-^{14}C, pyridoxine, and folic acid to alfalfa seedlings, stachydrine became radioactive whereas homostachydrine did not. Assuming that the enzymes controlling methylation of the nitrogen heterocycles are similar, this clearly indicates that only the stachydrine is being synthesized at a plant age of 2 to 3 weeks.

In the last of the experiments with radioactive lysine, the crude betaine hydrochlorides from the reineckate separation were recrystallized without the usual purification through a mercuric chloride complex. The first crop was expected to be homostachydrine hydrochloride (m.p. 217°), but it melted at 240–246°, and after several recrystallizations at 247–250° (decomp.). Elemental analysis of this hydrochloride indicated the formula $C_7H_7O_2N.HCl$, and the ultraviolet spectrum showed aromatic absorption. Since the substance was isolated from the stachydrine fraction from which the stronger bases had already been removed, it was assumed to be a betaine. Thus the choice of structure was reduced to one of the betaines of the three isomeric pyridinecarboxylic acids. Homarine, the α-isomer, was eliminated because its hydrochloride melts at 170° (15). Since the γ-isomer has not been found naturally, trigonelline, the β-isomer, seemed the more likely of the two remaining possibilities. The complete identity of the ultraviolet and infrared spectra, X-ray powder pattern and paper chromatographic properties for the natural sample, and synthetic trigonelline hydrochloride confirmed the structure. Synthetic trigonelline hydrochloride had m.p. 259–260° (decomp.) after softening at 257°, but the melting point of the natural material could not be raised above 250°. Other low-melting samples of trigonelline hydrochloride isolated from natural sources have been reported (16, 17, 18).

In the mercuric chloride precipitation normally used as a final purification step for stachydrine and homostachydrine, trigonelline does not precipitate with the reagent (20% hydrochloric acid saturated with mercuric chloride). Thus in previous work on alfalfa in this laboratory (1, 13, 14, 19) had trigonelline been present it would have remained unnoticed in the filtrate. To see if trigonelline is a normal constituent of the plant, alfalfa seedlings 16 days old and grown with the usual 30 g of seeds per tray (14, 19) were extracted, but trigonelline could not be detected. In the experiment described above in which trigonelline was isolated, 40 g of seeds per tray were used, and experience gained since suggests that this quantity overloads the trays so that although good germination occurred, some seeds did not grow because those already sprouting formed a light shield. This observation suggested that trigonelline may occur in alfalfa seeds and a direct extraction confirmed the idea. Indeed it is present in the betaine fraction of the seeds in a larger amount (38%) than stachydrine (33%) or homostachydrine (29%). It was not discovered in the extraction of seeds by Wiehler and Marion (1) because they used a final mercuric chloride purification. Furthermore, in the butanol – dilute hydrochloric acid system which they used for separating stachydrine (R_F 0.50) and homostachydrine (R_F 0.58) by paper chromatography, trigonelline has the same R_F as choline (0.41). Trigonelline (R_F 0.37) is readily separated from choline (R_F 0.57) in ethanol–ammonia (20). In this system homostachydrine has R_F 0.56 and stachydrine R_F 0.50.

Trigonelline and homostachydrine seem to be formed in alfalfa at the time of seed formation. The trigonelline stored in the seed is used up in the first few days of growth and the homostachydrine disappears between 1 and 5 months, since an extraction of

plants 5 months old showed stachydrine as the only betaine. Hence the biosynthesis of homostachydrine must take place during the flowering and seed-setting period. The proposed biosynthetic scheme remains as a working hypothesis, but no greater credence can be attached to it in view of recent findings that proline is not involved in the biosynthesis of stachydrine (21).

After removing the proteins in the alfalfa extractions the betaines were separated from the water-soluble residue by leaching with alcohol. The fraction insoluble in alcohol partly crystallized and the recrystallized compound was identified by its elemental analysis, infrared spectrum, and X-ray powder pattern as asparagine. The yield was 2.5% of the weight of the dry plant. Asparagine had been isolated from alfalfa previously (22) but the ease of isolation and the high yield in the present experiments are noteworthy.

EXPERIMENTAL

Melting points are corrected. The infrared absorption spectra were measured in nujol mulls on a Perkin–Elmer double beam spectrometer model 21B.

Feeding Experiments with Lysine-2-^{14}C

Alfalfa seeds were germinated on glass wool as previously described (14, 19) using 300 g in 10 trays (20×30 cm) for the first three experiments and 400 g for the fourth. It was found that discarding the nutrient solution and washing with distilled water each week (14, 19) made the plants too wet and was unnecessary for good growth to an age of 3 weeks.

Experiment 1.—On the 14th day 8.0 mg DL-lysine-2-^{14}C-monohydrochloride (purchased from Tracerlab Inc.) with a total activity of 3.2×10^7 d.p.m. in 500 ml nutrient solution was administered. Plants were harvested on the 20th day.

Experiment 2.—On the 14th day a mixture of 8.5 mg radioactive lysine, total activity 3.5×10^7 d.p.m., and 1 mg pyridoxine hydrochloride in 500 ml nutrient solution was administered. Plants were harvested on the 19th day.

Experiment 3.—On the 10th day a mixture of 7.6 mg radioactive lysine, total activity 3.0×10^7 d.p.m., 1 mg pyridoxine hydrochloride in nutrient solution, and 1 mg folic acid in sodium bicarbonate solution (23) was administered. Plants were harvested on the 20th day.

Experiment 4.—Nutrient solutions containing a total of 22 mg radioactive lysine, total activity 1.8×10^8 d.p.m., 3 mg pyridoxine, 5 mg folic acid in aqueous sodium bicarbonate, and 250 mg (±)-methionine were administered in three portions on the 7th, 11th, and 16th day. Plants were harvested on the 20th day.

Extraction Procedure (*cf. 1, 13, 14, 19*)

Preliminary defatting of the dried plant material by refluxing (4 hours) with petroleum ether (b.p. 75–90°) was advantageous in avoiding emulsions and giving sharper fractions in subsequent stages. This was particularly important during an extraction of alfalfa seeds. The plant material was boiled with three lots of 70% alcohol, but the extracts were not diluted with water (1) before precipitation of the proteins with lead acetate. After removing the excess of lead as sulphide, the filtrate was evaporated to dryness using octyl alcohol as required to prevent frothing. The alcohol-soluble substances in the resulting dark brown gum could not be obtained by direct extraction on account of the impermeable nature of the material. Of the several methods tried, the best was to dissolve the gum in an equal volume of hot water and slowly add 10 volumes of hot alcohol with vigorous swirling. Most of the material precipitated and after being cooled to 0° the

supernatant liquid was decanted. This process was repeated twice more. Frequently the gum which was precipitated on adding alcohol crystallized spontaneously, and after recrystallization of the solid from water, large white rhombs having m.p. 234° (decomp.) were obtained in yields from 2–3% of the dry plant weight. The compound was identified as (−)-asparagine hydrate by comparison of its infrared spectrum and X-ray powder pattern with those of an authentic specimen. Found for the anhydrous compound: C, 36.1; H, 6.0; N, 20.8. Calc. for $C_4H_8N_2O_3$: C, 36.4; H, 6.1; N, 21.2%; $[\alpha]_D^{25}$ −5.2° (*c*, 2.0 in water).

The choline and betaine reineckates were separated from the decanted alcoholic extract in the usual way (20, 24, 25, 26). A quicker method of obtaining the hydrochlorides from the reineckates than the precipitation technique (27) was ion exchange chromatography. Amberlite IRA 401 ion exchange resin was rinsed through two cycles with aqueous sodium hydroxide and hydrochloric acid. The chloride form was washed with methanol and a 50-ml tube was filled with the resin. The reineckate (0.50 g) was dissolved in boiling acetone (20 ml) and diluted with methanol (40 ml). The cooled solution was percolated rapidly through the column and eluted with methanol (100 ml), the operation being completed in 10 minutes. If slower flow rates were used, the absorbed reineckate ion began to decompose on the column, discharging an orange impurity. Removal of solvent from the eluate left a quantitative yield of the mixed betaine hydrochlorides, which were best separated by fractional crystallization. Trigonelline hydrochloride is far less soluble than the other two betaines so when present it was separated by using the minimum volume of boiling absolute ethanol for solution. The filtrate after removal of trigonelline was boiled, and successive additions of hot ethyl acetate gave fractions consisting of almost pure homostachydrine hydrochloride. The stachydrine remaining in the filtrate was very difficult to crystallize because contamination due to the homostachydrine still present rendered the mixture much more soluble than pure stachydrine hydrochloride. In the absence of trigonelline the usual mercuric chloride precipitation was very useful in purifying the betaine fraction.

Extraction Results

In the first three experiments with radioactive lysine, the average yield of betaine hydrochlorides was 0.35 g and the mixture was shown by paper chromatography to consist of stachydrine and homostachydrine in a ratio of *ca.* 1:1. In the fourth experiment, using 400 g of seeds, the yield of betaine hydrochlorides was 0.56 g. The first crystalline fraction (0.14 g) after further recrystallization from alcohol had m.p. 247–250° (decomp.). Found: C, 48.9; H, 4.8; N, 7.7; Cl, 20.3. Calc. for $C_7H_7NO_2$.HCl: C, 48.5; H, 4.65; N, 8.1; Cl, 20.4%. Ultraviolet spectrum in ethanol 0.2 *M* with respect to hydrochloric acid, λ_{max} 266 mμ, log ε 3.67. The ultraviolet and infrared spectra, X-ray powder pattern, and paper chromatograms of the natural sample were completely superimposable on those of a synthetic specimen of trigonelline hydrochloride, prepared by methylation of nicotinic acid according to the procedure of Cornforth and Henry (28). The synthetic material had m.p. 259–260° after softening at 257°. None of the above betaine fractions was radioactive.

R_F values of these compounds in butanol – concentrated hydrochloric acid – water (100:20:39) were: homostachydrine, 0.58; stachydrine, 0.50; trigonelline, 0.41; choline, 0.41; and in 95% ethanol – concentrated aqueous ammonia (95:5) were: homostachydrine, 0.56; stachydrine, 0.50; trigonelline, 0.37; choline, 0.57. Descending chromatography was used and the solvent front travelled *ca.* 18 inches, taking 40 hours in the acid system and 18 hours in the alkaline system. The spots were developed with a modi-

fied Dragendorff's reagent (29) in which the shade of color produced with fresh reagent was characteristic for these compounds. The reagent was made by dissolving potassium iodide (10 g) in water (10 ml) and glacial acetic acid (20 ml). Bismuth subnitrate (3.4 g) was dissolved in small portions with stirring. If necessary, the solution was filtered and then made up to 60 ml with water. For spraying, this stock solution (1 vol) was diluted with 30% acid (9 vol).

In the extraction of trigonelline from 300 g of alfalfa seeds, 0.52 g of betaine hydrochlorides was obtained (0.17% yield). By fractional crystallization 206 mg of trigonelline and 85 mg of homostachydrine hydrochlorides were separated. The remaining stachydrine fraction (0.23 g) was estimated by the intensity of spots on paper chromatograms to be one third homostachydrine, giving the following analysis of the betaine fraction: trigonelline, 38%; stachydrine, 33%; homostachydrine, 29%.

In the extraction of alfalfa 5 months old, 50 plants grown in soil were used, and the roots and aerial portions were treated separately. The former (dry weight 10 g) yielded 15 mg of stachydrine hydrochloride and the latter (dry weight 43 g) yielded 140 mg. No homostachydrine or trigonelline was detected by paper chromatography.

REFERENCES

1. WIEHLER, G. and MARION, L. Can. J. Chem. **36**, 339 (1958).
2. ROBERTSON, A. V. and MARION, L. Can. J. Chem. **37**, 829 (1959).
3. BEYERMAN, H. C. Rec. trav. chim. (In press).
4. GROBBELAAR, N. and STEWARD, F. C. J. Am. Chem. Soc. **75**, 4341 (1953).
5. LOWY, P. H. Arch. Biochem. Biophys. **47**, 228 (1953).
6. SCHWEET, R. S., HOLDEN, J. T., and LOWY, P. H. J. Biol. Chem. **211**, 517 (1954).
7. ROTHSTEIN, M. and MILLER, L. L. J. Am. Chem. Soc. **75**, 4371 (1953); J. Biol. Chem. **211**, 851 (1954).
8. MEISTER, A. and BUCKLEY, S. D. Biochim. et Biophys. Acta, **23**, 202 (1957).
9. MEISTER, A., RADHAKRISHNAN, A. N., and BUCKLEY, S. D. J. Biol. Chem. **229**, 789 (1957).
10. ZACHARIUS, R. M., THOMPSON, J. F., and STEWARD, F. C. J. Am. Chem. Soc. **76**, 2912 (1954).
11. MORRISON, R. M. Biochem. J. **50**, XIV (1952); **53**, 474 (1953).
12. ZACHARIUS, R. M., THOMPSON, J. F., and STEWARD, F. C. J. Am. Chem. Soc. **74**, 2949 (1952); **76**, 2908 (1954).
13. MORGAN, A. and MARION, L. Can. J. Chem. **34**, 1704 (1956).
14. WIEHLER, G. and MARION, L. J. Biol. Chem. **231**, 799 (1958).
15. HOPPE-SEYLER, F. A. Z. physiol. Chem. **222**, 105 (1933).
16. KNOWLES, W. S. and ELDERFIELD, R. C. J. Org. Chem. **7**, 389 (1942).
17. SCHOICHI YANAGITA. J. Pharm. Soc. Japan, **64**, 38 (1944).
18. OGATA, A. and KOMODA, T. J. Pharm. Soc. Japan, **63**, 653 (1943).
19. LEETE, E., MARION, L., and SPENSER, I. D. J. Biol. Chem. **214**, 71 (1955).
20. BREGOFF, H. M., ROBERTS, E., and DELWICHE, C. C. J. Biol. Chem. **205**, 565 (1953).
21. ROBERTSON, A. V. and MARION, L. Can. J. Chem. (In press).
22. VICKERY, H. B. J. Biol. Chem. **60**, 647 (1924); **61**, 117 (1924).
23. LASCELLES, J., CROSS, M. J., and WOODS, D. D. J. Gen. Microbiol. **10**, 267 (1954).
24. STRACK, E. and SCHWANEBERG, H. Z. physiol. Chem. **245**, 11 (1936).
25. GLICK, D. J. Biol. Chem. **156**, 643 (1944).
26. LEE KUM-TATT. Nature, **182**, 655 (1958).
27. KAPFHAMMER, J. and BISCHOFF, C. Z. physiol. Chem. **191**, 179 (1930).
28. CORNFORTH, J. W. and HENRY, A. J. J. Chem. Soc. 601 (1952).
29. PALMER, K. H. (Private communication).

THE ACTION OF SODIUM IODIDE ON *O*-*p*-TOLUENE-SULPHONYL-1,4;3,6-DIANHYDRIDES OF D-MANNITOL, D-GLUCITOL, AND L-IDITOL[1]

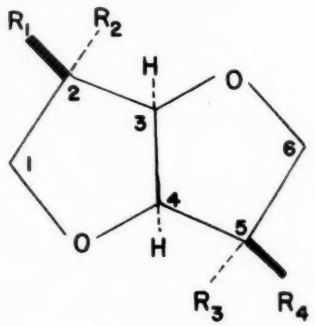
M. JACKSON AND L. D. HAYWARD

ABSTRACT

In the displacement reaction with sodium iodide in acetic anhydride solution the rates of reaction of four isohexide tosyl esters were compared to that of an *O*-acetyl-*O*-tosylisosorbide recently described. The tosyloxy group in the latter compound was replaced rapidly and was therefore in the 5-*endo*-position, the compound being correctly named 2-*O*-acetyl-5-*O*-*p*-toluenesulphonyl-1,4;3,6-dianhydro-D-glucitol. The product of the reaction was isolated as a crystalline material, and was identified as 2-*O*-acetyl-5-deoxy-5-iodo-1,4;3,6-dianhydro-L-iditol.

INTRODUCTION

The 1,4;3,6-dianhydrides of D-mannitol, D-glucitol, and L-iditol (isomannide (I), isosorbide (II), and isoidide (III)) represent a useful series of compounds for the study of reaction mechanisms from a steric point of view. These compounds possess two fused five-membered rings having a *cis*-arrangement of the ring junction. The rings, being nearly planar, are inclined to one another at an angle of about 120°. Substituents on carbon atoms 2 and 5 can either be on the outside of the V formed by the rings when they are called *exo* (R_2, R_3) or on the inside and named *endo* (R_1, R_4). The conformation of the ring system is rigid in as much as it cannot undergo inversion of the type occurring in cyclohexane conformations and the shape and dimensions of the molecules are therefore fairly well known (2).



I	R_1 = OH	R_2 = H	R_3 = H	R_4 = OH
II	H	OH	H	OH
III	H	OH	OH	H
IV	OTs	H	H	OTs
V	H	OTs	H	OTs
VI	H	OTs	OTs	H
VII	H	OTs	I	H
VIII	H	O.CO.CH_3	H	OTs
IX	H	O.CO.CH_3	I	H
X	H	I	I	H

[1]*Manuscript received March 2, 1959.*
Contribution from the Department of Chemistry, University of British Columbia, Vancouver, B.C.

Can. J. Chem. Vol. 37 (1959)

The reaction of sodium iodide with the isohexide di-*O*-*p*-toluenesulphonates (di-*O*-tosylates) has been studied on a qualitative basis by several workers. Hockett *et al.* (3, 4) prepared the 2,5-dideoxy-2,5-diiodo derivative from isomannide di-*O*-tosylate (IV) and a monodeoxy-monoiodo derivative from the isosorbide isomer (V). Matheson and Angyal (5) compared the reaction of these compounds with that of other secondary tosylates by isolating the sodium *p*-toluenesulphonate formed but not the other organic products. These authors found that, in the D-mannitol derivative (IV), both tosyloxy groups are readily replaceable by iodine, but in the D-glucitol derivative (V) only one, and in the L-iditol derivative (VI) neither, tosyloxy group was replaceable. Wiggins and Wood (6), by isolating the dianhydro products, obtained a parallel series of results from reactions of the di-*O*-tosyl and di-*O*-mesyl derivatives with sodium iodide and with lithium chloride respectively. These results confirm that both sulphonoxy groups of the isomannide derivatives and one in the isosorbide derivatives are readily replaced by halide ion, whereas the second group of the isosorbide derivatives and both groups of the isoidide derivatives failed to react under the same conditions.

This behavior was explained (5) by an S_N2 mechanism with attack by the halide ion at the face of the carbon atom opposite the sulphonoxyl group. It may be seen from models that this mode of attack is hindered by the ring system when the group to be displaced is in the *exo* position, as in isoidide derivatives and the 2-position of isosorbide. When the group is *endo*, however, the attack is relatively easy and this situation is found in isomannide derivatives and the 5-position of isosorbide. Other reactions in the isohexide series having an S_N2 mechanism show a similar selectivity (7).

Cope and Shen (1) recently described the preparation of a crystalline *O*-acetyl-*O*-tosyl isosorbide by partial tosylation of the parent compound (II) followed by acetylation. The 2-position was assigned to the *O*-tosyl group since it was considered probable that the *exo*-C_2-hydroxy group was preferentially tosylated.

In the present work the rates of the reaction with sodium iodide of the di-*O*-tosyl isohexides (IV, V, and VI) and of the 2-*O*-tosyl-5-deoxy-5-iodoisoidide (VII) obtained from V were compared to that of the *O*-acetyl-*O*-tosylisosorbide of Cope and Shen in order to show the position of the *O*-tosyl group in the latter compound. Both the sodium *p*-toluenesulphonate and the isohexide products were isolated.

RESULTS AND DISCUSSION

The tosyl esters were treated in acetic anhydride solution at reflux temperature with sodium iodide in the ratio of 1.2 moles of iodide to 1 of *O*-tosyl group. The reaction was stopped at 2, 4, and 8 hours and, after cooling, the precipitated sodium *p*-toluenesulphonate was filtered off and weighed. The yields of the salt obtained are shown in Fig. 1.

The rate curve for the replacement of the tosyloxy group in the *O*-acetyl-*O*-tosylisosorbide closely followed those for the reaction of an *endo* group (IV and one tosyloxy group in V) whereas it was distinctly different from those of the *exo*-tosyl groups (VI and VII). Thus the tosyloxy group in this compound must occupy an *endo* position. In isosorbide this is found at the 5-position and the compound prepared by Cope and Shen (1) was therefore 2-*O*-acetyl-5-*O*-*p*-toluenesulphonyl-1,4;3,6-dianhydro-D-glucitol (VIII).

Confirmation of the rate study came from the isolation in high yield (87%) of a crystalline iodo-compound from the reaction products. This compound melted at 44.0–44.5° C and analyzed correctly for a deoxyiodo-*O*-acetylisohexide. The infrared spectrum con-

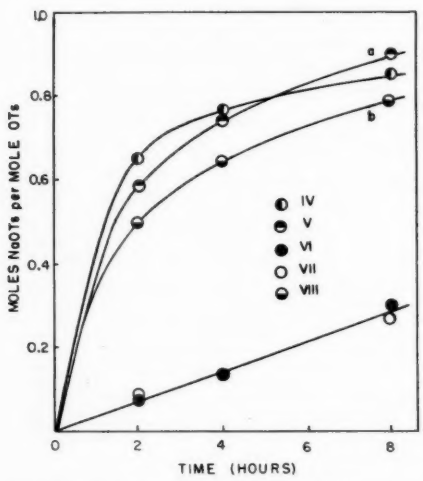


FIG. 1. Rates of replacement of tosyloxy groups by iodine in *O*-tosylisohexides. In acetic anhydride at reflux temperature with 1.2 moles sodium iodide per mole *O*-tosyl group.
(*a*) Sodium iodide, 1.2 moles per mole V; yields calculated for one *O*-tosyl group. With 2.5 moles sodium iodide per mole V the curve was displaced upwards due to the slow reaction of the second tosyloxy group.
(*b*) Acetyldeoxyiodoisoidide isolated in 74% yield.

firmed the presence of the *O*-acetyl group and of the tetrahydrofuran rings. The isoidide configuration was assigned to this compound from comparison with other reactions in this series and the compound was therefore 2-*O*-acetyl-5-deoxy-5-iodo-1,4;3,6-dianhydro-L-iditol (IX).

Cope and Shen (1) found that treatment of the *O*-acetyl-*O*-tosylisosorbide with sodium *t*-butoxide gave a good yield of 1,4;2,5;3,6-trianhydro-D-mannitol and it was proposed that this reaction took place via a normal anhydro-ring-formation reaction, i.e. by an internal displacement of the tosyloxy group by the alkoxide ion formed from the hydrolysis of the *O*-acetyl group. This mechanism is not possible with the *O*-tosyl group in the *endo* position and the *O*-acetyl in the *exo* position. The reaction must therefore proceed via the initial hydrolysis of the *O*-tosyl group followed by the displacement of the *O*-acetyl group or by some transesterification mechanism of the type proposed by Cope and Shen (1).

EXPERIMENTAL

Reaction of the p-Toluenesulphonate Esters of the Isohexides with Sodium Iodide

The sulphur contents, melting points, and specific rotations of the esters together with the reported constants are shown in Table I.

The *O*-tosyl compound (0.005 mole) was dissolved in acetic anhydride (dried and redistilled, 25 ml) and sodium iodide (A.R. grade, 0.0062 or 0.0124 mole) added. The mixture was refluxed for 2 hours, cooled, and left to stand for 30 minutes. The crystalline precipitate was collected by filtration into a weighed sintered glass funnel. The precipitate was washed with acetic anhydride (10 ml) and dried at 100° C to constant weight. The filtrate and washings were combined, refluxed for a further 2 hours, and the precipitate collected as before. The solution from this stage was refluxed for 4 hours before isolation of the final precipitate and the isohexide products.

TABLE I

p-Toluenesulphonate esters of the 1,4;3,6-dianhydrohexitols (isohexides)

Isohexide	Substituents		For-	% S		Found		Reported		
	C_2	C_5	mula	Calc.	Found[a]	m.p. (° C)[b]	$[\alpha]_D^{22c}$	m.p. (° C)	$[\alpha]_D$	Reference
Isomannide	OTs	OTs	IV	14.1	13.8	90–91	+95.5	93–94	+98.5	(8)
								89–90	+98.5	(5)
Isosorbide	OTs	OTs	V	14.1	13.6	100–101	+57.4	101–102	+57.8	(5)
								98.3–99.6	+57.4	(3)
Isoidide	OTs	OTs	VI	14.1	13.9	105–105.5	+33.5	90	+38.2	(9)
								105.5–106	+33.2	(6)
								106	+33	(5)
Isoidide	OTs	I	VII[d]	7.52	7.6	82–83	+56.5	90.8–91.3	+52.7	(3)
Isosorbide	OAc	OTs	VIII[e]	9.35	9.2	64–65	+77.9	64–65	+50.5	(1)

[a]Determined by "oxygen flask" method of Lysyj and Zarembo (10).
[b]Compounds were recrystallized from methanol to constant melting points, which are uncorrected.
[c]In chloroform, *c*, 1.1 to 2.0.
[d]Prepared from the reaction of 2.4 moles of sodium iodide on V.
[e]Prepared by partial tosylation of isosorbide following the method of Cope and Shen (1).

After excess acetic anhydride was removed by distillation under reduced pressure, the reaction mixture was shaken with water and chloroform and the aqueous layer was washed with further amounts of chloroform. The combined chloroform extracts were washed with saturated sodium bicarbonate solution, sodium thiosulphate solution (10%), and water, then dried, and evaporated. The resulting syrups crystallized after rubbing at 0° C.

2-O-Acetyl-5-deoxy-5-iodo-1,4;3,6-dianhydro-L-*iditol* (IX)

Treatment of 2-*O*-acetyl-5-*O*-*p*-toluenesulphonyl-1,4;3,6-dianhydro-D-glucitol (2-*O*-acetyl-5-*O*-tosylisosorbide) (VIII) with 1.5 mole excess of sodium iodide as described above gave an 87% yield of crystalline compound. With a 0.24 mole excess of sodium iodide the yield was 74%. Recrystallization several times from methanol gave a product melting at 44.0–44.5° C (uncorr.), $[\alpha]_D^{22}$ +100.5° (*c*, 0.83 in chloroform). Anal. Calc. for a mono-*O*-acetyl-monodeoxy-monoiodoisohexide, $C_8H_{11}IO_4$: C, 32.23; H, 3.72; I, 42.59%. Found: C, 32.46; H, 3.57; I, 42.38%.

The infrared spectrum of the compound (KBr window) showed absorption at 1240, 1370, and 1746 cm^{-1} corresponding to the acetyl group and at 845, 874, and 930 cm^{-1} corresponding to the tetrahydrofuran rings (11).

2,5-Dideoxy-2,5-diiodo-1,4;3,6-dianhydro-L-*iditol* (X)

This compound was isolated from the reaction of 2,5-di-*O*-tosylisomannide (IV) with 0.24 mole excess of sodium iodide. After several recrystallizations from methanol it melted at 65–66° C (uncorr.) and had $[\alpha]_D^{22}$ +110° (*c*, 0.645 in chloroform). Hockett and co-workers (4) had reported m.p. 61–62° C; Wiggins and Wood (6) m.p. 61–63° C, $[\alpha]_D^{19}$ +107.9° (*c*, 1.97 in chloroform).

ACKNOWLEDGMENT

One of us (M.J.) is grateful to the Powell River Company Limited, Powell River, B.C., for the award of a scholarship.

REFERENCES

1. COPE, A. C. and SHEN, T. Y. J. Am. Chem. Soc. **78**, 5912 (1956).
2. MILLS, J. A. Advances in carbohydrate chemistry. Vol. 10. Academic Press, Inc., New York. 1955. p. 46.

3. HOCKETT, R. C., FLETCHER, H. G., SHEFFIELD, E. L., and GOEPP, R. M. J. Am. Chem. Soc. **68**, 927 (1946).
4. HOCKETT, R. C., FLETCHER, H. G., SHEFFIELD, E. L., GOEPP, R. M., and SOLTZBERG, S. J. Am. Chem. Soc. **68**, 930 (1946).
5. MATHESON, N. K. and ANGYAL, S. J. J. Chem. Soc. 1133 (1952).
6. WIGGINS, L. F. and WOOD, D. J. C. J. Chem. Soc. 1180 (1951).
7. COPE, A. C. and SHEN, T. Y. J. Am. Chem. Soc. **78**, 3177 (1956).
8. MONTGOMERY, R. and WIGGINS, L. F. J. Chem. Soc. 393 (1946).
9. BASHFORD, V. G. and WIGGINS, L. F. J. Chem. Soc. 371 (1950).
10. LYSYJ, I. and ZAREMBO, J. E. Anal. Chem. **30**, 428 (1958).
11. BARKER, S. A. and STEPHENS, R. J. Chem. Soc. 4550 (1954).

IONIZATION POTENTIALS OF METHYLAMINES AND ETHYLAMINES BY ELECTRON IMPACT[1]

JACQUES COLLIN

ABSTRACT

Recent determinations of the ionization potentials of methylamines and ethylamines obtained by electron impact have been compared with photoionization results and discussed in relation to the Franck–Condon principle. It seems that the observed discrepancies are mainly due to differences in the sensitivities of the detecting devices.

Six different sets of data on the ionization potentials of aliphatic amines have been published, showing a surprising spread of values going from 0.68 v for methylamine, to 1.60 v for triethylamine, which is much higher than the expected errors of such determinations.

We have therefore remeasured these ionization potentials with a Nier-type mass spectrometer[2] using the semilog method of interpreting the results (1).

TABLE

Ionization potentials of methylamines and ethylamines (in ev)

	Photon impact		Electron impact				
Molecule	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)	(*f*)	This work
Ammonia	10.15	—	—	10.52	—	—	10.34±0.07*
Methylamine	8.97	—	9.8	9.41	9.41	9.65	9.41±0.08
Dimethylamine	8.24	—	9.6	—	9.21	9.5	8.93±0.03
Trimethylamine	7.82	—	9.4	—	9.02	9.2	8.32±0.02
Ethylamine	8.86	<8.8†	—	9.32	—	9.6	9.19±0.05
Diethylamine	8.01	—	—	—	—	9.5	8.44±0.01
Triethylamine	7.50	—	—	—	—	9.1	7.85±0.07

*The errors indicated are precision errors; they only give an idea of the reproducibility of the measurements. The absolute errors can not, of course, be given since no definite interpretation can be given as yet.

†Estimated adiabatic ionization potential; the estimated vertical ionization potential given by the authors is 9.5 v.

(*a*) Watanabe, K. J. Chem. Phys. **26**, 542, 1773 (1957).

(*b*) Hurzeler, H., Inghram, M. G., and Morrison, J. D. J. Chem. Phys. **28**, 76 (1958).

(*c*) Sugden, T. M., Walsh, A. D., and Price, W. C. Nature, **148**, 373 (1941).

(*d*) Morrison, J. D. and Nicholson, A. J. C. J. Chem. Phys. **20**, 1021 (1952).

(*e*) Omura, Higasi, and Baba. Bull. Chem. Soc. Japan, **29**, 504 (1956).

(*f*) Collin, J. Bull. soc. chim. Belges, **62**, 411 (1953).

Our values as well as all previously existing data are given in the table, and the following may be said about them:

1. Our new electron impact results are markedly lower than previous data obtained by the same technique, with the exception of methylamine. All ionization curves were very steep and their initial curvature was practically the same as that of the reference rare gases. This is shown by the fact that the results obtained by taking the 0.1% of 50-v abundance value or the 1% of 50-v abundance value are the same within 0.03 v with the exception of methylamine using krypton as a reference gas, where the difference was 0.08 v.

2. Our results are considerably higher than the photon impact values and the explana-

[1]*Manuscript received November 28, 1958.*
Contribution from Institut de Chimie Générale, Université de Liège, 1b, Quai Fr. Roosevelt, Liège, Belgium.
[2]*These measurements were performed in the laboratories of the National Research Council, Ottawa, Canada.*

tion of the important differences observed is not straightforward. The significance of the values depends mainly on the relative position and shape of the potential energy surfaces for the ion and the molecule. If the internuclear distances are the same for both species no such discrepancies should be observed, both photon and electron impact processes being of the Franck–Condon type. This is, however, no more the case if the equilibrium distances are not the same, the discrepancies arising then from the differences in the sensitivities of the detecting devices. The problem is to find the reasons why such modification of internuclear distances occur in the ion.

It is theoretically thought (2) that the ionization of amines proceeds by removal of a non-bonding electron of the nitrogen lone pair. Another possibility is, however, that the ionization is due to the removal of a σ-bonding electron, as has recently been suggested by Hurzeler, Inghram, and Morrison (3) on the basis of their differential interpretation of photon impact data. These authors have also shown that no adiabatic ionization potential could be obtained from the direct experimental ionization curves, for ethylamine and *n*-propylamine. This would explain the literature discrepancies. Since the C–N, C–C, and C–H bond energies are very much the same in amines and hydrocarbons, however, it does not seem that the removal of a bonding electron from the C–N bond would be so much easier in amines than in hydrocarbons (where for instance $I(C_2H_6)$ = 11.76 v and $I(C_3H_8)$ = 11.21 v (4)). The same argument may be applied to the removal of a bonding electron from a C–H or N–H bond, which must have rather higher ionization potentials since $I(CH_4)$ = 13.12 v (4) on one hand, and since in ammonia the corresponding ionization potential is 15.31 v (5) on the other hand. Moreover the small variation of the ionization potential with the length of the hydrocarbon chain in aliphatic amines (6) compared to a considerable one in hydrocarbons (4) is rather in favor of an ionization process on the nitrogen atom.*

In any case, however, the ionization must bring about a change in the state of hybridization of the nitrogen atom which might be responsible for the modification of internuclear distances, and thus of the potential energy surfaces.

The great variation of ionization potentials with substitution on the nitrogen remains to be explained. It should be remembered that the hybridized valence state of the nitrogen atom in these molecules may be represented by the formulas

$$\text{I} \qquad [\text{N}(1s)^2(2s + \delta 2p)^2(2p + \delta' 2s)^2]$$

and

$$\text{II} \qquad [\text{N}(1s)^2(2s + \delta 2p)^2(2p + \delta' 2s)^2(2p + \delta'' 2s)].$$

State I applies to NH_3 and NR_3 types, with different values of the δ parameter according to the nature of the substituent radical R, and state II applies to NRR_2' types, such as encountered in primary and secondary amines, the values of the δ parameters depending again on the nature of R and R′. Therefore, according to the nature of the radicals R, the non-bonding N atomic orbital $(2s+\delta 2p)$ will have different p character and its energy will not be the same. This is the case for the series NH_3, RNH_2, R_2NH, R_3N and the ionization potentials are expected to vary.

It does not seem probable that electrostatic factors arising from the existence of important dipole moments in those molecules may play a significant role in the ionization processes.

**The cause of the considerable differences between mass spectrometric results, although not evident, should probably mainly be attributed to differences in sensitivity of the mass spectrometers used, as indicated above, and, to a certain extent, to the interpretation of the results by different methods. However, the general trend of variation of ionization potentials with hydrocarbon chain length, as indicated in the work of Morrison and Nicholson, is still of application, notwithstanding the correctness of the absolute values.*

3. Except for ethylamine and *n*-propylamine where the photon impact evidence seems conclusive (3) and brings information on the relative positions of the potential surfaces for the ground molecular and ionic states, no definite conclusions as to the real meaning of the available data can be given. It seems, however, that electron impact values, being considerably higher than photoionization ones, refer to vertical transition to a vibrationally excited ion; the true reason for the modification of internuclear distances in the ion is not evident.

REFERENCES

1. LOSSING, F. P., TICKNER, A. W., and BRYCE, W. A. J. Chem. Phys. **19**, 1254 (1951).
2. MULLIKEN, R. S. J. Chem. Phys. **3**, 584 (1935).
3. HURZELER, H., INGHRAM, M. G., and MORRISON, J. D. J. Chem. Phys. **28**, 76 (1958).
4. HONIG, R. E. J. Chem. Phys. **16**, 105 (1948).
5. MCDOWELL, C. A. J. Chem. Phys. **24**, 618 (1956).
6. MORRISON, J. D. and NICHOLSON, A. J. C. J. Chem. Phys. **20**, 1021 (1952).

THE PROTON RESONANCE SPECTRA OF FURAN AND PYRROLE[1]

R. J. Abraham and H. J. Bernstein

ABSTRACT

Interpretation of the fine structure of the NMR spectra of some five-membered "aromatic" heterocyclic ring compounds indicates that spin coupling constants between adjacent protons on the ring and those across the ring are approximately equal. This is in contrast to the situation in six-membered rings where coupling constants between protons ortho to one another are much larger than those in the meta position.

Quadrupole effects of the N^{14} nucleus in pyrrole broaden the NH signal but do not interfere with the spin coupling of the NH proton with other protons. The surprising result is obtained that the spin couplings of the NH proton with α and β protons in the ring are very nearly equal in magnitude.

The analysis and interpretation of the high resolution proton magnetic resonance spectra of non-benzenoid aromatics have been largely neglected. Corey *et al.* (1) have shown that the magnetic resonance spectra of natural compounds containing furan rings are of considerable help in determining the structure of these compounds, as the position of substitution in the furan ring can be found by inspection of the chemical shift data. Anderson (2) has measured the coupling constants and chemical shifts for 2-bromo-5-chlorothiophen, but no systematic high resolution studies have so far been reported for 5-membered ring compounds.

It is the purpose of this work to present the complete analysis of the spectra of the heterocyclic molecules furan and pyrrole and also to investigate the effect of substitution on the magnitude of the spin–spin coupling constants in these molecules. We shall show that the coupling constants obtained by the analysis of these spectra are very different from those found for benzene systems.

For the analysis of these complex spectra we have employed two simplifying techniques. Firstly, that of substitution to reduce the number of interacting protons: deuterium substitution is preferable as the magnetic constants remain unchanged, but methyl substitution can also be used in many cases since it only changes the coupling constants by a small amount. Secondly, by observing the spectrum of the molecule dissolved in a suitable solvent: very often one group of protons may be more affected by medium effects than the others and this may increase the chemical shift difference between the groups and simplify the fine structure considerably. In particular, the intensity distribution becomes more amenable to simple analytical treatment. This process does not appear to affect the size of the spin coupling constants appreciably, only the chemical shifts. Also, these solution spectra help in an understanding of the nature of the molecular complexes formed in solution and some preliminary investigations of these effects have been made here.

EXPERIMENTAL

The spectra were measured with a Varian V-4300 high resolution spectrometer, incorporating a field stabilizer and operating at 40 Mc/s. A spinning sample tube of 5 mm outside diameter was used. The standard side-band technique was employed to measure the signal separations.

[1]*Manuscript received December 1, 1958.*
Contribution from the Division of Pure Chemistry, National Research Council, Ottawa, Canada. This paper was presented at the Symposium on Molecular Structure, Columbus, Ohio, June 1958.
Issued as N.R.C. No. 5178.

The chemical shifts, expressed here in c.p.s. relative to water as an external reference, were measured with a dioxane or cyclohexane reference capillary, adding 58 c.p.s. to the values measured relative to dioxane, and 142 c.p.s. to those measured relative to cyclohexane.

The accuracy of the chemical shift measurements was estimated to be ± 1 c.p.s. and of the coupling constants, ± 0.1 c.p.s.

The furan compounds used are the commercially available samples. The methylated pyrroles were kindly supplied by Dr. E. Bullock and the deuterated pyrrole by Dr. L. Leitch.

RESULTS AND DISCUSSION

(*a*) *Furans*

We shall consider first the spectra of furan and its derivatives. These spectra are simplified by the large chemical shift difference between the 2,5 and 3,4 protons of the ring due to the strongly electronegative oxygen atom. (This numbering procedure is much more convenient than using α and β when discussing derivatives.)

The spectra of furan and 2-furfurol together establish completely the assignments and coupling constants in these compounds. The spectrum of furan (Fig. 1) consists of two

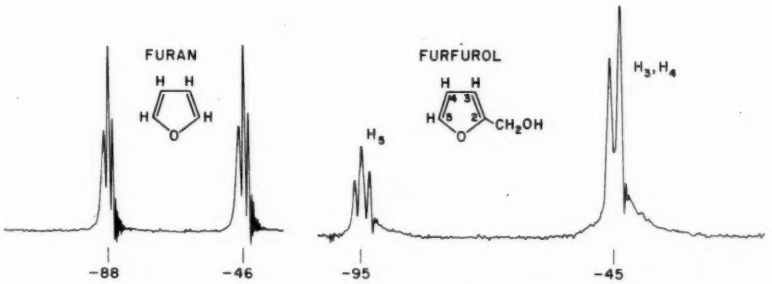


FIG. 1. The proton magnetic resonance spectra of liquid furan and furfurol. The spectrum of the CH_2OH group is not shown. In all figures the direction of increasing applied field is to the right.

widely separated triplets of equal intensity in agreement with earlier observations (1), due obviously to the 2,5 and 3,4 protons of the ring. The spectrum of the ring protons of 2-furfurol (Fig. 1), in which the 2 proton has been replaced by the $—CH_2OH$ group, consists of a low field triplet and a high field doublet of twice the intensity. The intensity distribution immediately shows that the 5 proton is to low field.

The spectrum of furan is of the type A_2X_2 (the nomenclature and full treatment of such systems follows that of Pople, Bernstein, and Schneider (3)). From this analysis, the triplet structure of the component groups in furan can arise when $J_{23} = J_{24}$. The spin coupling constant is then given by the triplet separation and is equal to 1.3 c.p.s. This is confirmed by the spectrum of 2-furfurol. The doublet pattern of the 3 and 4 protons and the triplet structure of the 5 proton are to be expected if $J_{23} = J_{24}$, and from the spectrum the coupling constant is found to be equal to that in furan (1.3 c.p.s.).

Thus in these compounds the adjacent or ortho coupling constant (J_{23}) is almost the same as the cross ring or meta coupling constant (J_{24}), in contrast to the situation in benzene derivatives, where the ortho, meta, and para proton coupling constants are about 8, 2, and 0.5 c.p.s. respectively (4).

The methylene and hydroxyl peaks of 2-furfurol are not shown in Fig. 1. They are to high field and are single peaks. Presumably any coupling between the methylene and hydroxyl proton is quenched by the rapid exchange of the hydroxyl proton. There is also no coupling between the methylene and the ring protons. This is in contrast to the behavior of other groups in this position (see below). Also the $—CH_2OH$ group does not alter the chemical shift of the 3 proton with respect to the 4 proton of the ring. This is again in contrast to the behavior of other groups in this position. For example, in the case of 2-methyl furan and 2-furfural, the 3 and 4 protons have different chemical shifts, giving rise to an ABX-type spectrum (5), and also the 3 proton spin couples with the protons of the substituent group to further complicate the spectrum.

The spectrum of 2-methyl furan is shown in Fig. 2. On the basis of the chemical shift data obtained for furan and 2-furfurol, we assign the low field peak to the 5 proton. This

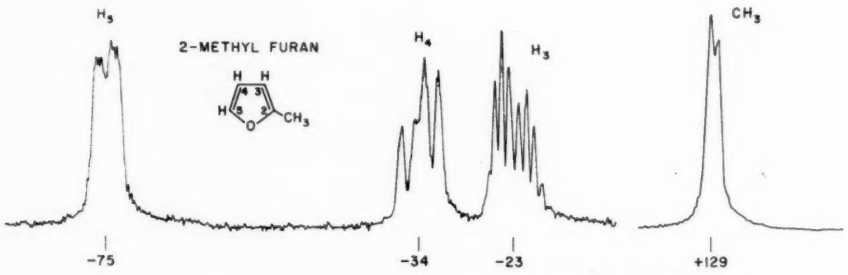


FIG. 2. The proton magnetic resonance spectrum of liquid 2-methyl furan.

is reasonable as the methyl group would not be expected to have any great effect on the electron distribution in the ring. Of the remaining peaks, one of the normal quartet patterns typical of each proton in an ABX-type spectrum (5) is split into more lines by interaction with the methyl group. This group of signals is assigned to the 3 proton since it is reasonable to expect strongest coupling with the proton nearest to the methyl group. The methyl group interaction splits each of the four original lines of this signal into quartets (intensity ratio 1:3:3:1) but the weaker lines will not be observed except on the extreme edges of the signal, so that the main part can be regarded as a quartet split into doublets. However, the splitting due to the methyl is equal to that between the outside pairs of the original quartet so that some overlapping occurs, and thus the complete signal consists of two main triplets with two weak outer lines. The value of the spin coupling constant between CH_3 and H_3 is given directly by the doublet splitting of the methyl group of 0.9 c.p.s. The calculated energies and intensities (i.e. peak heights) for this spectrum are shown along with the observed ones in Table I. Protons 4, 3, and 5 correspond to A, B, and X respectively. Transitions occurring with less than 0.3 c.p.s. separation have been grouped together, as this represents the limit of resolution of the instrument. The intensities for the B group have been normalized separately. The agreement is well within the experimental limits of accuracy. The chemical shifts and spin coupling constants derived from this analysis are given in Table II. The low field signal of proton 5 is not resolved completely into the expected four components, but is more complex and unresolved. This is probably due to a very small interaction of the methyl group with this proton via the oxygen atom. This interaction would be expected to be less than 0.2 c.p.s. and therefore below the limits of resolution of the instrument.

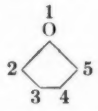
TABLE I

Observed and calculated spectra for α-methyl furan

Transition	Origin	Energy (c.p.s.) Calc.	Energy (c.p.s.) Obs.	Relative intensity Calc.	Relative intensity Obs.
1	*B*	0	0	.09	.17
2	*B*	.9	.9	.37	.41
3	*B*	1.1			
4	*B*	1.8	1.8	.56	.56
5	*B*	2.0			
6	*B*	2.7	2.8	.53	.50
7	*B*	2.9			
9	*B*	3.1			
8	*B*	3.8	4.1	.72	.67
10	*B*	4.2			
11	*B*	4.0			
12	*B*	5.1	5.2	.94	.82
13	*B*	4.9			
14	*B*	6.0	6.0	.63	.61
15	*B*	5.8			
16	*B*	6.9	6.7	.16	.22
17	*A*	13.0	13.2	1.26	1.11
18	*A*	14.7	14.7	1.26	1.21
19	*A*	16.1	16.1	.74	.75
20	*A*	17.4	17.6	.74	.71
21	*X*	53.2	53.7	1	1.29
22	*X*	54.8		1	
23	*X*	54.2	55.7	1	1.21
24	*X*	55.8		1	

TABLE II

Chemical shifts and coupling constants for furans (shift relative to water (c.p.s.))



	J_{45}	J_{25}	J_{24}	α	β			
Furan	1.3	1.3		−88	−46			
Furfurol	1.3	1.3		−91	−45	OH +4		CH_2 +38
2-Methyl furan	1.7	1.1	3.5	−75	−34, −23		CH_3 +129	
Furfural	1.5	0.9	3.4	−94	−76, −48		CHO −169	
5-Chlormethyl furfural*			3.8		−76, −50		CHO −166	CH_2 +30

*A bulk susceptibility correction of +15 c.p.s. has been added to the chemical shifts. This value was obtained by a comparison of the chemical shifts in furfural and 5-chlormethyl furfural referred to an internal standard.

The spectrum of furfural is shown in Fig. 3. In this spectrum both the individual groups and their component lines are well separated and thus the formal analysis is quite straightforward. However, the complete assignment cannot be made. The spectrum is an *ABX*-type spectrum modified by the coupling of the aldehyde proton to one of the ring protons. This gives rise to the extra fine structure of the low field signal. The coupling constant

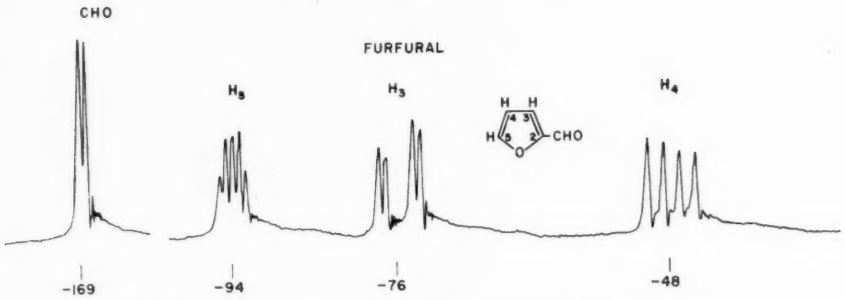


FIG. 3. The proton magnetic resonance spectrum of liquid furfural.

with the aldehyde proton is approximately equal to the spacing in the unperturbed quartet. This results in a quintet of intensity ratio 1:2:2:2:1. The coupling constant is given directly by the size of the aldehyde doublet splitting of 0.7 c.p.s. The remaining chemical shifts and coupling constants for this spectrum are now easily calculated.

However, the assignment of the groups in this spectrum is not clear. On the basis of the chemical shift data, the low field signal would be expected to be that of proton 5 and Corey *et al.* have given this assignment. Further, spin–spin coupling constants are known to be very insensitive to substitution. For example, Richards *et al.* have shown that the coupling constants in a series of para-substituted benzenes vary by less than 10% over a whole range of substituent groups (6). This again supports the assignment of the low field signal in furfural to proton 5 as this would make the coupling constant J_{34} equal to 3.4 c.p.s., in excellent agreement with the value of 3.5 c.p.s. for α-methyl furan.

However, the spin–spin coupling of 0.7 c.p.s. between the aldehyde proton and the proton giving the low field signal would normally be expected to be between the aldehyde proton and the nearest neighbor proton in the ring. On this basis the low field signal would be assigned to proton 3.

This question of the assignment of the low field signal was finally resolved experimentally with the aid of the magnetic resonance spectrum of liquid 5-chlormethyl furfural. In this spectrum the signals of the H_3 and H_4 protons appeared as a quartet (AB group, J = 3.8 c.p.s.) and the low field signal of the ring proton spectrum of furfural was absent. This identifies the low field signal with H_5 and further, neither the H_3 proton nor the aldehyde proton showed any signs of fine structure. Thus the surprising result is obtained that fine structure of the aldehyde signal in furfural arises from spin–spin interaction of the aldehyde proton with H_5. The assignments and coupling constants for furfural and 5-chlormethyl furfural are given in Table II. Protons 3 and 4 in furfural are assigned on the basis of the similarity of the coupling constants in furfural to those in α-methyl furan, i.e. the smallest coupling constant is taken as J_{35}. This agrees with the chemical shift data as hydrogen 3 in furfural would be expected to be at lower field than hydrogen 4 due to the influence of the electronegative aldehyde group.

One of the most significant results emerging from this study is the low value of the coupling constants between adjacent protons in these compounds. There are two of these coupling constants, J_{23} and J_{34}, with values of approximately 2 and 3 c.p.s. respectively, in contrast to the value of 8 c.p.s. found in benzene derivatives. These low values we shall show are found also in the pyrrole ring and although thiophen gives a complex and as yet not analyzed spectrum, in 2-bromo-5-chlorothiophen, J_{34} equals 3.9 c.p.s. which is of the

same magnitude as the above values. Thus the magnitude of the ortho coupling appears to be a function of ring size. This is similar to the behavior in the aromatic hydrocarbon rings (7).

(*b*) *Pyrroles*

The spectrum of pyrrole (Fig. 4) consists of a very broad line at low field (not shown in the figure) due to the hydrogen in the NH group (8), and a complex eight line portion

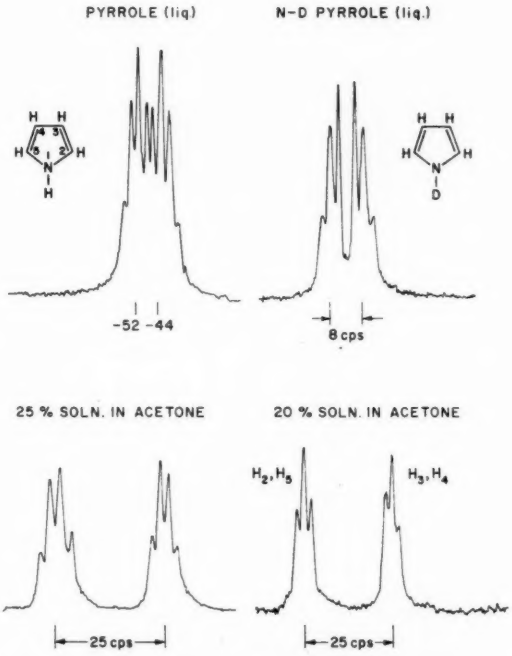


FIG. 4. The proton magnetic resonance spectra of pyrrole and N–D pyrrole. The N–H peak is at low field and not shown.

due to the ring hydrogens. The complete analysis of this A_2B_2X-type spectrum cannot be undertaken without some further experimental results. These were obtained by considering the spectra of pyrrole solutions and also pyrrole derivatives.

The chemical shift difference between the 2,5 and 3,4 protons of pyrrole increases when pyrrole is dissolved in acetone or carbon tetrachloride and the spectrum now splits into two quartets with the 1 proton line shifting to much lower fields and broadening so as to be almost undetectable, but the spacing between the lines in each quartet does not change. Although dilution has effected a considerable simplification in the spectrum, this spectrum can still be interpreted in many ways and the final solution was arrived at by considering the spectra of some methyl- and deuterium-substituted pyrroles. It is apparent that the spectrum of N-deuteropyrrole dissolved in acetone (Fig. 4) is exactly the same as that of furan. This means that in the pyrrole ring as in the furan ring, the ortho coupling constant (J_{23}) equals the meta or cross ring coupling constant (J_{24}) and is equal to the observed separation in the triplets of 1.9 c.p.s. The correct intensity

pattern for this type of spectrum (triplet ratios 1:2:1) is observed only in the solution case, where the chemical shift is large compared with the spin coupling constant. In the spectrum of the pure liquid these intensities are altered appreciably due to the decrease in the chemical shift, although the separations between the lines remain constant. The spin coupling constants in N-deuteropyrrole must be equal to those in pyrrole and the separation between the lines of the quartets in the solution spectrum of pyrrole is indeed almost equal to this coupling. However, the quartet character in pyrrole must arise from another interaction, namely, the coupling of proton 1 with the other ring protons. In order to obtain the observed spectrum, proton 1 must couple equally to both protons 2 and 3 of the ring and this coupling must be equal to the quadruplet spacing of 2.1 c.p.s. This unexpected result was verified by considering the spectra of 2,3- and 2,5-dimethyl pyrrole (Fig. 5). In the former, protons 4 and 5 will each have a triplet fine structure due

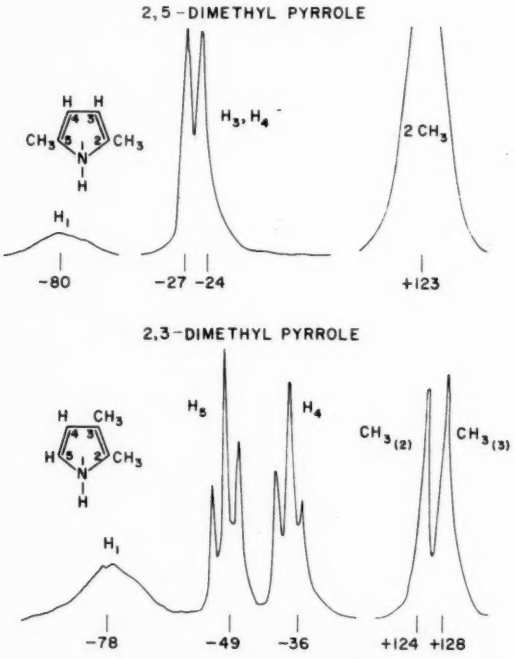


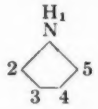
FIG. 5. The proton magnetic resonance spectra of liquid 2,3- and 2,5-dimethyl pyrrole.

to the equal coupling between themselves and proton 1, and in the latter, protons 3 and 4 will be a doublet whose splitting will give directly the coupling constant J_{13} ($=J_{14}$). The coupling constants obtained from these spectra are close to those in pyrrole (see Table III). Note that the splitting due to the spin coupling with the methyl groups is too small to be detected in these compounds. The chemical shift values for these compounds also show that in the pyrroles, as in the furans, the α protons lie to low field (Table III).

The spectrum of 2-methyl pyrrole (Fig. 6) is complicated, as is that of 2-methyl furan, by the different chemical shifts of protons 3 and 4 and the interaction of the methyl

TABLE III

Chemical shifts and coupling constants for pyrroles (shift relative to water (c.p.s.))



Compound*	J_{45}	J_{35}	J_{34}	J_{15}	J_{14}	N–H	α	β	$(CH_3)_\alpha$	$(CH_3)_\beta$
Pyrrole	2.1	2.1		2.1	2.1	−80	−52	−44		
N–D Pyrrole	2.0	2.0								
α-Methyl pyrrole	2.6	1.5	2.8	2.4	2.8	−80	−52	−43, −34	+124	
50% Acetone soln.	2.6	1.5	2.8	2.4	2.8		−49	−33, −24	+125	
2,3-Dimethyl pyrrole	2.6			2.6	2.6	−78	−49	−36	+124	+128
50% Acetone soln.	2.6			2.6	2.6	−123	−47	−25	+130	+132
2,4-Dimethyl pyrrole						−66	−42	−25	+121	+126
2,5-Dimethyl pyrrole					2.7	−80		−25	+123	
Krypto pyrrole						−62	−42		+121	+125

*The spectrum of the pure liquid is given unless otherwise stated.

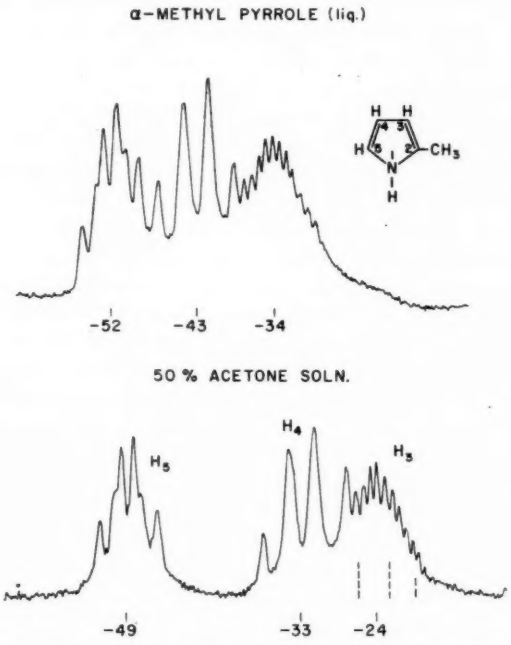


FIG. 6. The proton magnetic resonance spectrum of α-methyl pyrrole. The spectrum of the methyl group is to high field and not shown.

group with proton 3. Although there are several possible assignments for this spectrum, the information obtained from the other pyrrole spectra provides the following means of interpreting this spectrum completely. The low field peak is assigned to proton 5 since the influence of the methyl group on the electron distribution in the ring is small and therefore the order of the chemical shifts would be expected to be the same as in pyrrole.

The sextet fine structure of this peak results if two of the three coupling constants involved (J_{15}, J_{35}, and J_{45}) are equal. In pyrrole all these constants are equal but in this case we assume that the cross ring coupling J_{35} has decreased whilst the others remain constant. This identifies all the three coupling constants immediately. The assignment for the other two protons is now straightforward, the high field peak is identified unambiguously as that of proton 3 by its complex structure, and the separation of the component lines gives the coupling constant $J_{H_3.CH_3}$, which is equal to 0.6 c.p.s. The quartet structure of proton 4 shows immediately that the three coupling constants (J_{14}, J_{34}, and J_{45}) are all equal. The average values for the coupling constants are given in Table III.

Two other substituted pyrroles were examined, 2,4-dimethyl pyrrole and krypto (3-ethyl-2,4-dimethyl) pyrrole. The ring proton peaks of these substances would be expected to be two triplets and a doublet respectively. In fact, they are spread out into broad humps in each case. This may well be due to some extra splitting due to interaction with the methyl groups, which would be expected to be small and therefore unresolvable.

The results of these investigations in the pyrroles are given in Table III. The coupling constants obtained show again the same characteristics as in the furan ring. The adjacent or ortho coupling (J_{23}) is about the same as the cross ring or meta coupling (J_{24}) although slightly larger than in furan. Also there is some change in the coupling constants even with methyl substitution. There are further effects to be noted here, in particular, the size of the interaction between proton 1 and the ring protons and the almost equal values of J_{12} and J_{13}. The peak for proton 1 is broadened by the quadrupole coupling of the N_{14} nucleus into a very broad single line, though it has been reported that the triplet structure characteristic of the N_{14} interaction does appear at higher temperatures (8). However, the quadrupole coupling has no effect upon the coupling of the NH hydrogen to the ring protons. This is a similar situation to that found in formamide (9).

The invariance of these coupling constants when the compound is dissolved in various solvents is well demonstrated in pyrrole. The spacing between the individual lines in the pyrrole quartets does not change when pyrrole is dissolved in carbon tetrachloride, acetone, methanol, or even glacial acetic acid, although the chemical shift separation between the quartets does change appreciably. Pyrrole is known to form weak hydrogen bonds with itself (10) (although there is still some doubt as to the exact nature of these bonds), and also with solvents like acetone, and thus the observed chemical shift changes in this solvent are very probably due to this effect. However, this hydrogen bonding does not appear to affect the value of the coupling constant between the bonded proton and the other ring protons. Also it can be shown that this hydrogen bonding is not accompanied by any rapid exchange process. The spectrum of pyrrole in methanol at 50° C has still the same pattern as at room temperature although that of the methanol consists only of two sharp peaks, since the rapid exchange of the hydroxyl proton removes any fine structure associated with it. To remove a coupling of about 2 c.p.s., proton 1 would have to have a lifetime shorter than about 0.1 second. Thus the lifetime of proton 1 is certainly longer than 0.1 second, which means the exchange rate is slow under these conditions. This proton, however, can be exchanged, since pyrrole was deuterated in this position using heavy water, although this required many processes before the exchange was complete.

NOTE ADDED IN PROOF

The spectra of furan, furfurol, and pyrrole have been interpreted on the basis that $J_{35} = J_{45}$. This is not the only interpretation of these spectra and a more detailed

analysis (to be presented in a subsequent publication) shows that the correct coupling constants differ slightly from the values quoted above and in particular the two coupling constants are not equal. The observed splitting in these spectra instead of being equal to J_{35} or J_{45} is equal to $(J_{35}+J_{45})/2$.

REFERENCES

1. COREY, E. J., SLOMP, G., DEV, S., TOBINAGA, S., and GLAZIER, E. R. J. Am. Chem. Soc. **80** (No. 5), 1204 (1958).
2. ANDERSON, W. A. Phys. Rev. **102**, 151 (1956).
3. POPLE, J. A., BERNSTEIN, H. J., and SCHNEIDER, W. G. Can. J. Chem. **35**, 1487 (1957).
4. GUTOWSKY, H. S., HOLM, C. H., SAIKA, A., and WILLIAMS, G. A. J. Am. Chem. Soc. **79**, 4596 (1957).
5. BERNSTEIN, H. J., POPLE, J. A., and SCHNEIDER, W. G. Can. J. Chem. **35**, 65 (1957).
6. RICHARDS, R. E. and SCHAEFFER, T. P. Trans. Faraday Soc. **54**, 1280 (1958).
7. SCHNEIDER, W. G., BERNSTEIN, H. J., and POPLE, J. A. J. Am. Chem. Soc. **80**, 3497 (1958).
8. ROBERTS, J. D. J. Am. Chem. Soc. **78**, 4495 (1956).
9. PIETTE, L. H., RAY, J. D., and OGG, R. A., JR. J. Mol. Spectroscopy, **2**, 66 (1958).
10. JOSIEN, M. L., PINEAU, P., PATY, M., and FUSON, N. J. Chem. Phys. **24**, 1261 (1956).

THE BIOGENESIS OF ALKALOIDS

XXII. SOME INTERMEDIATE STEPS IN THE TRANSFORMATION OF TRYPTOPHAN TO GRAMINE[1]

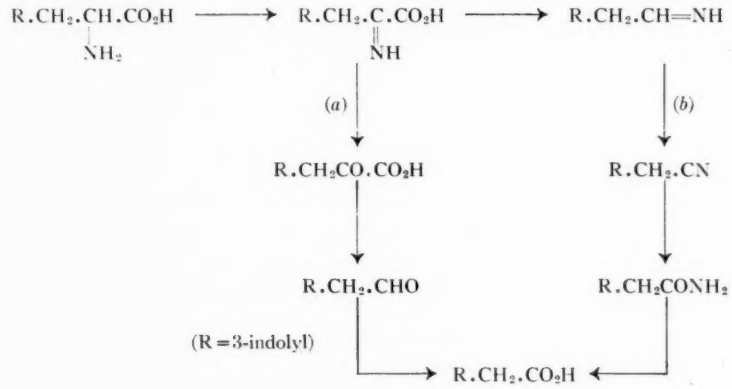
ALBERTO BRECCIA[2] AND LÉO MARION

ABSTRACT

In germinating barley it is known that tryptophan is converted into gramine. In an attempt to find the intermediate steps in this conversion, it has been established that neither 3-indolylacetic acid, 3-indolylglyoxylic acid, 3-indolylaldehyde, nor 3-indolylacetamide (all labelled with ^{14}C in the side chain) was a precursor of gramine. It has been shown, however, that both 3-indolylpyruvic acid and 3-indolylacrylic acid gave rise to radioactive gramine when fed to germinating barley. It is likely that these two acids constitute two intermediates in the pathway tryptophan–gramine.

It has been established earlier (1) that the precursor of the alkaloid gramine is tryptophan, and that the biogenesis involves scission of the side chain of the amino acid not at the indole nucleus as might have been expected, but between the α- and β-carbons (i.e., $R—CH_2—CH(NH_2)CO_2H$). We have now attempted to establish some of the intermediate steps involved in the transformation.

The metabolism of tryptophan, not involving destruction of the indole system, can take place according to Gordon (2) via two potential pathways:



In support of pathway (*a*) Wildman, Ferri, and Bonner (3) had presented evidence that 3-indolylpyruvic acid might be an intermediate in the formation of indolylacetic acid. As a result of paper chromatographic studies, the presence of indolylpyruvic acid has been reported in immature maize kernels (4) and in maize endosperms (5). It has been shown since, however, that indolylpyruvic acid is destroyed on the paper chromatogram under the conditions employed (6) whereas the substance described by Stowe and Thimann (5) is stable, and consequently could not have been indolylpyruvic acid (6).

Furthermore, according to the work of Spenser, Crawhall, and Smyth (7), who have

[1]*Manuscript received February 27, 1959.*
Contribution from the Division of Pure Chemistry, National Research Council, Ottawa, Canada.
Issued as N.R.C. No. 5186.
[2]*National Research Council of Canada Postdoctorate Fellow, 1957–58.*

studied the oxidative decarboxylation of amino acids, it is improbable that indolylpyruvic acid can be an intermediate in the conversion of tryptophan to indolylacetaldehyde.

That indolylacetaldehyde is an immediate precursor of indolylacetic acid in one pathway is indicated by the fact that when homogenates of *Phaseolus mungo* are exposed to X rays the oxidase is blocked, and the aldehyde accumulates in the homogenate while the production of indolylacetic acid decreases (2).

Concerning the second pathway (*b*), indolylacetonitrile has been reported to occur in nature (8, 9, 10), and Stowe and Thimann (11) have shown that *Avena* coleoptiles convert indolylacetonitrile into the corresponding acid.

The metabolism proceeds further, and it has been considered that 3-indolylacetic acid is converted to 3-indolylaldehyde (12) presumably via indolylglyoxylic acid. Manning and Galston (13) have shown, however, that in the presence of indolylacetic acid oxidase from pea seedlings, indolylacetic acid undergoes oxidative conversion into products which do not include 3-indolylaldehyde. They also showed that the aldehyde does not participate as an intermediate in the formation of the final products. On the other hand, their work did not preclude the possibility that in certain plants the metabolism of indolylacetic acid could be via indolylglyoxylic acid to indolylaldehyde, and it was conceivable that the acid and its metabolic products might be intermediates in the conversion of tryptophan to gramine.

When, however, 3-indolylacetic-α-^{14}C acid was fed to germinating barley under conditions identical with those previously described (14), the gramine isolated from the plants showed no radioactivity whatsoever. It was found that most of the radioactivity remained in the nutrient solution (pH 7). The small quantity of radioactivity that became incorporated into the barley was mostly found in the fraction of the extract that contained the acidic and neutral compounds. It thus appeared that the administered indolylacetic acid must have been destroyed very early in the experiment by the action of light or possibly by an oxidase that could be present in the nutrient solution. Indeed, it has been found that 3-indolylacetic acid (100 mg) added to the nutrient solution (100 ml) left after the plants had been harvested was completely destroyed in 3 hours.

In further experiments, 3-indolylglyoxylic-α,β-^{14}C acid was fed to germinating barley, both with and without ascorbic acid, which is known to act as an antagonist of oxidases. The gramine isolated failed to show any radioactivity. 3-Indolylaldehyde (labelled on the aldehydic carbon) was also fed to germinating barley, alone and also in the presence of ascorbic acid. In each case, however, the gramine isolated from the harvested plants was inactive.

In order to test the other possible pathway, 3-indolylacetamide-α-^{14}C was also fed to germinating barley, but with the same negative result.

Unless the negative results described be attributable to the failure of the labelled substances to penetrate into the plant, these results indicate that the conversion of tryptophan to gramine must proceed via a pathway that does not involve 3-indolylacetic acid. It was therefore supposed that the amination necessary for the formation of gramine took place before the rupture of the side chain, i.e., on an indolyl compound still containing a 3-carbon side chain. Such a supposition is supported by the fact that β-(3-indolyl)-acrylic acid and β-(3-indolyl)-lactic acid are known to occur in barley and other plants (9). Although the occurrence of 3-indolylpyruvic acid in plants has not been satisfactorily demonstrated (6), it has been assumed that this acid could be one of the precursors of gramine.

In subsequent experiments 3-indolylpyruvic-β-^{14}C acid was administered to germinating

barley and the gramine was isolated from the plant after a few days as usual. The gramine thus obtained was radioactive (1.18×10^5 counts per minute per mmole); it was fused with potassium hydroxide and the resulting indolylcarboxylic acid was decarboxylated. The carbon dioxide evolved (isolated as barium carbonate) contained all the radioactivity originally present in the gramine. Hence there has been no randomization and it can be concluded that, as observed previously with tryptophan (1, 14) the β-carbon of the indolylpyruvic acid is still present in the gramine, and that the scission of the side chain of the acid occurred between the α- and β-carbons.

Experiments in which 3-indolylacrylic-β-^{14}C acid was fed to germinating barley were also performed. The results were similar to those obtained with indolylpyruvic acid, and the gramine isolated from the plants was radioactive. The radioactivity again was located on the CH_2 of the gramine, as shown by the results of the degradation of the alkaloid.

EXPERIMENTAL

Preparation of the Labelled Compounds

The indolyl derivatives were prepared by slight modifications of known methods. A sample of each compound was chromatographed on paper to make sure of its purity.

Radioactive gramine was synthesized from polyoxymethylene-^{14}C first hydrolyzed to formaldehyde and then condensed with indole and dimethylamine as described by Kuhn and Stein (15). The yield of radioactive gramine based on polyoxymethylene was 80%. Sp. act. 5.26×10^7 c.p.m. per mmole.

Indolyl-3-aldehyde was prepared via the methosulphate of gramine labelled with ^{14}C in the CH_2 of the side chain by the method of Thesing (16). Using smaller quantities of tetrahydrofuran and glacial acetic acid than those specified resulted in an increased yield of aldehyde, m.p. 196°. Yield 55%. Sp. act. 4.12×10^7 c.p.m. per mmole.

3-Indolylglyoxylic acid was prepared by the condensation of indole with diethyl-oxalate-1,2-^{14}C (17) obtained by esterification of silver oxalate-1,2-^{14}C with ethyliodide. Sp. act. 3.89×10^7 c.p.m. per mmole.

3-Indolylacrylic-β-^{14}C acid was prepared by condensation of radioactive indolylaldehyde with ethyl malonate in pyridine containing a small quantity of piperidine according to the well-known method. The acid, m.p. 198–200 (decomp.), was obtained in 48.2% yield and 20% of the aldehyde used was recovered. The specific activity of the 3-indolyl-acrylic acid was 4.32×10^7 c.p.m. per mmole. A paper chromatogram of the acid with the solvent benzene – propionic acid – water (100:70:5) was developed during 12 hours and brought out with a 1% solution of *p*-dimethylaminobenzaldehyde in *N* hydrochloric acid. It showed two spots, one with the R_f of the acid, the other with the same R_f as 3-indolylaldehyde. The radioactivity of the aldehyde spot was 100 counts per minute, and that of the acid spot was 1.0×10^4 counts per minute. Hence the radioactivity of the product is 99% due to the acid and 1% due to the aldehyde still present.

3-Indolylpyruvic-β-^{14}C acid was prepared from radioactive indolylaldehyde as described by Ellinger and Matsuoka (18). Yield 50.3%, m.p. 205–210° (decomp.). Sp. act. 5.04×10^7 c.p.m. per mmole.

3-Indolylacetamide-α-^{14}C was obtained from the action of potassium cyanide on radioactive gramine as described by Snyder and Pilgrim (19). Yield 50.4% based on the gramine, m.p. 149–150°. Sp. act. 4.30×10^7 c.p.m. per mmole.

Administration of the Radioactive Compound to the Barley

A 700-g sample of Charlottetown No. 80 barley was evenly divided among twelve 20×25 cm Pyrex glass trays. Distilled water (50 ml) was added to each of the trays,

which were placed in a closed germination cabinet. The barley was watered daily. On the sixth day of sprouting, a solution of the labelled substance was added to each of the trays, and the barley allowed to grow for a further 5 days, all as previously described (20).

The barley was harvested on the 11th day of sprouting, the shoots worked up as described before (14), and the gramine isolated.

Because of the low solubility of 2-indolylaldehyde in water, this substance (32 mg, total activity 4.48×10^6 c.p.m.) was dissolved in 65% ethanol (10 ml) and the solution added to the trays of growing barley. After a lapse of 8 hours, distilled water (600 ml) was gradually added to the trays over a period of 6 hours.

The 3-indolylpyruvic acid (50 mg) used in the feeding experiments had a specific activity of 5.04×10^7 c.p.m. per mmole, and the gramine (121 mg) isolated had an activity of 1.18×10^5 c.p.m. per mmole.

The 3-indolylacrylic acid (71 mg) fed to the barley had an activity of 4.32×10^7 c.p.m. per mmole, and the gramine obtained from the plants treated with this acid had a specific activity of 1.86×10^5 c.p.m. per mmole.

Degradation of Gramine

(*a*) Some of the radioactive gramine obtained from the plants was converted to the methiodide and treated with sodium methoxide as described by Madinaveitia (21). The reaction was carried out as in previous cases (14) and the liberated dimethylamine isolated as ethyldimethylamine picrate showed no radioactivity.

(*b*) A quantity of gramine (13 mg) of specific activity 1.38×10^4 c.p.m. per mmole was mixed with inert gramine (150 mg) and fused with potassium hydroxide (3 g) as described previously (14) and the resulting 3-indolylcarboxylic acid was heated at 190–230° in a stream of nitrogen. The stream of nitrogen and evolved gas was bubbled through a barium hydroxide solution, and the precipitated barium carbonate was filtered and dried. It had a specific activity of 1.36×10^4 c.p.m. per mmole.

DISCUSSION

Since neither 3-indolylglyoxylic acid, 3-indolylaldehyde, nor 3-indolylacetamide are precursors of gramine in barley, it is not surprising that as now shown, and as Henry and Leete (22) have recently reported, 3-indolylacetic acid should also fail to act as a precursor of gramine. Unless it be assumed that each of these four labelled substances was destroyed in the nutrient solution instead of penetrating into the plant, as indeed indolylacetic acid was, then it must be concluded that the pathway in the transformation of tryptophan to gramine is not via indolylacetic acid.

On the other hand, both 3-indolylpyruvic acid and 3-indolylacrylic acid are precursors of gramine. Since the alkaloid, obtained from barley fed with indolylacrylic acid, was more strongly radioactive than that isolated from barley fed with indolylpyruvic acid, it is probable that indolylpyruvic acid is the precursor of indolylacrylic acid. It is assumed, therefore, that in the first step of the biogenesis tryptophan is converted by transamination to 3-indolylpyruvic acid from which at a further stage in the same pathway 3-indolylacrylic acid is formed. Experiments are underway to attempt to determine at which stage amination occurs.

REFERENCES

1. LEETE, E. and MARION, L. Can. J. Chem. **31**, 1195 (1953).
2. GORDON, S. A. *In* Wain, R. L. and Wightman, F. The chemistry and mode of action of plant growth substances. Butterworth Scientific Publications, London. 1956. p. 66.

3. WILDMAN, S. G., FERRI, M. G., and BONNER, J. Arch. Biochem. **13**, 131 (1947).
4. KEFFORD, N. P. J. Exptl. Botany (London), **6**, 129 (1955).
5. STOWE, B. B. and THIMANN, K. V. Nature, **172**, 764 (1953).
6. BENTLEY, J. A., FARRAR, K. R., HOUSLEY, S., SMITH, G. F., and TAYLOR, W. C. Biochem. J. **64**, 44 (1956).
7. SPENSER, I. D., CRAWHALL, J. C., and SMYTH, D. G. Chem. & Ind. 796 (1956).
8. VON DENFFER, D., BEHRENS, M., and FISCHER, A. Naturwiss. **39**, 550 (1952).
9. VLITOS, J. and MEUDT, W. Contribs. Boyce Thompson Inst. **17**, 197 (1953).
10. BLOMMAERT, K. L. J. Nature, **174**, 970 (1954).
11. STOWE, B. B. and THIMANN, K. V. Arch. Biochem. Biophys. **51**, 449 (1954).
12. VON DENFFER, D. and FISCHER, A. Naturwiss. **39**, 549 (1952).
13. MANNING, D. T. and GALSTON, A. W. Plant Physiol. **30**, 225 (1955).
14. BOWDEN, K. and MARION, L. Can. J. Chem. **29**, 1037 (1951).
15. KÜHN, H. and STEIN, O. Ber. **70**, 567 (1937).
16. THESING, T. Chem. Ber. **87**, 507 (1954).
17. ELKS, J., ELLIOTT, D. F., and HEMS, B. A. J. Chem. Soc. 629 (1944).
18. ELLINGER, A. and MATSUOKA, Z. Z. physiol. Chem. **109**, 259 (1920).
19. SNYDER, H. R. and PILGRIM, F. J. J. Am. Chem. Soc. **70**, 3770 (1948).
20. KIRKWOOD, S. and MARION, L. Can. J. Chem. **29**, 30 (1951).
21. MADINAVEITIA, J. J. Chem. Soc. 1927 (1937).
22. HENRY, D. W. and LEETE, E. Abstracts, 134th Am. Chem. Soc. Meeting. Chicago. 1958. p. 43e.

THE NATURE OF PYRACONITINE[1]

D. J. McCaldin[2] and Léo Marion

ABSTRACT

A new investigation of pyraconitine and pyraconine has confirmed the presence of a skeletal carbonyl in these compounds. Reduction of pyraconitine with sodium borohydride produces a hydrolytic base which no longer contains a carbonyl group. The Wolff–Kishner reaction reduces the carbonyl in pyraconitine, eliminates the elements of methanol, and hydrogenates the resulting double bond as well as hydrolyzing off the benzoyl ester. These observations indicate the presence in aconitine of the sequence $R_2C(OAc).CHOH.CH(OCH_3)$-. On the basis of the structure of aconine previously derived from the X-ray structure of demethanolaconinone the foregoing results determine the skeletal position occupied by the labile acetoxy group in aconitine.

It has long been known (1) that aconitine ($C_{34}H_{47}O_{11}N$) when pyrolyzed eliminates acetic acid and gives rise to pyraconitine ($C_{32}H_{43}O_9N$). The loss of acetic acid has been variously interpreted as producing an epoxide (1, 2), an ethylenic linkage (3), or a carbonyl group (4). The last interpretation was based on the presence of a broad absorption band in the infrared spectrum of pyraconitine and a weak band in the spectrum of pyraconine hydrochloride. The ethylenic linkage suggested by Schneider (3) would, on the basis of his proposed structure for aconitine, require that the salts of pyraconitine be anhydronium salts, and neither the hydrochloride of pyraconitine nor that of its hydrolytic product pyraconine show the characteristic absorption of anhydronium salts in the infrared, so that this suggestion can be discarded. A new investigation of pyraconitine has therefore been undertaken, and the results obtained confirm the presence of a carbonyl in the pyrolytic products and permit the location of the acetoxy group in the carbon–nitrogen skeleton of the alkaloid.

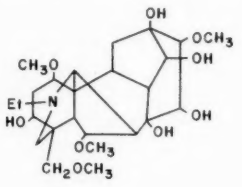
Crystalline pyraconitine was prepared by heating aconitine to 185–190°. Its saponification product, pyraconine ($C_{25}H_{39}O_8N$), was isolated as the crystalline hydrochloride. The infrared spectrum of pyraconitine contained a split absorption band at 1696–1717 cm^{-1} due to the benzoyl ester carbonyl and a second carbonyl in a six-membered ring. The latter was confirmed by the presence of a weak band at 1709 cm^{-1} in the infrared spectrum of pyraconine, which no longer contains the benzoyl group. In order to ascertain whether this weak carbonyl band was significant a solution of pyraconine in chloroform, of known concentration, was used for the infrared spectrum. The carbonyl band had an extinction coefficient $\epsilon^{(a)}_{max}$ 300, thus showing it to be a true carbonyl absorption band, since the absorption is significant when $\epsilon^{(a)}_{max} > 200$ (5). Furthermore, this absorption peak no longer showed in the infrared spectrum of the Wolff–Kishner reduction product of pyraconitine. On treatment with sodium borohydride, pyraconitine underwent hydrolysis as well as reduction of the carbonyl group to a hydroxyl and gave rise to a compound, $C_{25}H_{41}O_8N$, which in the infrared showed no absorption in the region 1600–1850 cm^{-1}. Had the reaction not involved the reduction of a skeletal carbonyl the product should have been identical with pyraconine, but it proved to be quite different. These results, therefore, confirm the conclusion of Shima and Amiya (4) that the elimination of acetic acid in the pyrolysis of aconitine is accompanied by the formation of a carbonyl group.

[1]*Manuscript received March 18, 1959.*
Contribution from the Division of Pure Chemistry, National Research Council, Ottawa, Canada.
Issued as N.R.C. No. 5190.
[2]*National Research Council of Canada Postdoctorate Fellow.*

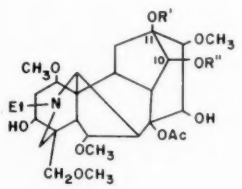
The Wolff–Kishner product of pyraconitine had the empirical formula $C_{24}H_{39}O_6N$, and contained only three methoxyl groups. Hence the reaction involved not only the reduction of the carbonyl to a methylene and saponification of the benzoyl group as expected; it also involved the loss of methanol and the hydrogenation of the resulting double bond. The absence of a double bond was evidenced by the infrared spectrum, which contained no absorption bands characteristic of such a function, and also by the attempted catalytic hydrogenation, which yielded the unchanged material. The Wolff–Kishner reaction is known to follow such a course when the carbonyl is α to a methoxyl, although in some cases the product is unsaturated (6).

In order that the elimination of acetic acid might give rise to a ketone it is necessary that the acetoxy group in aconitine be vicinal to a secondary hydroxyl, and to account for the behavior of pyraconine in the Wolff–Kishner reduction it is also necessary that this hydroxyl be vicinal to a methoxy group. It must be concluded, therefore, that aconitine contains the sequence of substituents $R_2C(OAc).CH(OH).CH(OCH_3)$- and that pyraconine contains the sequence $R_2CH.CO.CH(OCH_3)$-, which in the Wolff–Kishner reduction product becomes $R_2CH.CH_2.CH_2$-.

From the X-ray crystallographic study of demethanolaconinone hydriodide trihydrate (7) it follows that the structure of aconine is that represented by formula I. If we assume that no ring enlargement accompanies the pyrolytic reaction, then structure I presents



I



II

only one choice for the location of the acetoxy group in aconitine whose structure is shown by formula II where either R′ or R″ = C_6H_5CO and the other is H. It has previously been shown that the secondary hydroxyl in ring A is unsubstituted (8) and hence the benzoyl ester group can only be in either position 10 or 11 (cf. for numbering see ref. 8). It is hoped from current work to determine which of these two positions is occupied by the benzoyl ester group.

Aconitine possesses many points of similarity with delphinine. Like it, it possesses a bicyclo(1,2,3)octane system (9) which, however, contains one more hydroxyl group due to which the pyrolytic derivatives obtained from the two alkaloids are of a different nature. Jacobs and Pelletier (10) have shown that in delphinine it is a secondary hydroxyl on a five-membered ring that is esterified with benzoic acid. On the assumption that a similar situation exists in aconitine, the structure II (R′ = H, R″ = COC_6H_5) is preferred.

EXPERIMENTAL

All infrared absorption spectra were measured on nujol mulls, unless otherwise mentioned, with a Perkin–Elmer double beam spectrometer Model 21B. The melting points were determined on a Kofler hot stage and were not corrected.

Pyraconitine

Aconitine (1 g) was heated at 185–190° (metal bath) in a slow stream of nitrogen until effervescence ceased. The pale yellow liquid was cooled and dissolved in the minimum quantity of hot acetone. Hexane was added dropwise until a slight turbidity was observed. After some time pyraconitine crystallized as colorless needles (510 mg), m.p. 167–168° (literature gives 167.5° (1)). It had $[\alpha]_D^{24}$ −103° (*c*, 1.21 in ethanol) and pK_a 7.72 (determined in 50% ethanol). Found: C, 65.26; H, 7.21. Calc. for $C_{32}H_{43}O_9N$: C, 65.62; H, 7.46%. Infrared spectrum: 1696 cm^{-1} (skeletal carbonyl), 1717 cm^{-1} (ester carbonyl), the hydriodide crystallized as pale yellow rosettes from acetone–ether, m.p. 218–220°. Infrared spectrum: 1720 cm^{-1} (broad) due to ester and skeletal carbonyl groups.

Pyraconine

Pyraconitine was saponified with alcoholic sodium hydroxide and the product isolated as the hydrated hydrochloride, which separated from water as colorless prisms, m.p. 135° (literature (2) gives m.p. 135°), $[\alpha]_D^{22}$ −126.7° (*c*, 1.20 in water). Infrared spectrum: 1709 cm^{-1} (six-membered ring carbonyl).

Wolff–Kishner Reduction of Pyraconitine

Pyraconitine (230 mg) was dissolved in triethylene glycol (3 ml) and 95% hydrazine (2.5 ml) added to the solution. The reaction mixture was maintained at 150° for 1 hour under an atmosphere of nitrogen. Solid potassium hydroxide (1 g) was then added to the mixture and the temperature raised and maintained at 185° for a further hour. The dark reaction mixture was cooled and water (10 ml) was added. The aqueous solution was then extracted with six 20-ml portions of ether and the combined extract dried over magnesium sulphate. Evaporation of the ether extract yielded a gum (105 mg) that failed to crystallize, but formed a perchlorate that crystallized from a mixture of acetone and ether as colorless prisms, m.p. 258–259° (decomp.), $[\alpha]_D^{22}$ +61.9 (*c*, 1.25 in ethanol). Found: C, 53.70; H, 7.05; OMe, 17.03. Calc. for $C_{24}H_{39}O_6N.HClO_4$: C, 53.55; H, 7.26; $3OCH_3$, 17.36%.

Sodium Borohydride Reduction of Pyraconitine

Pyraconitine (370 mg) was dissolved in 80% methanol (10 ml) and sodium borohydride (150 mg) was added to the solution. After 1 hour, the solution was evaporated *in vacuo* and the residue dissolved in water (5 ml). The solution was extracted with five 10-ml portions of chloroform and the dried extract evaporated. There was left a gummy residue (280 mg) which formed a hydrochloride that crystallized from aqueous acetone as colorless prisms. The melting point was variable but fusion started at 155–156°, $[\alpha]_D^{21.5}$ −23.4 (*c*, 2.70 in water). Found (sample dried at 50° for 2 hours): C, 54.57; H, 8.27. Calc. for $C_{25}H_{41}O_8N.HCl.2H_2O$: C, 54.10; H, 8.27%. Found (sample dried at 100° at 0.1 mm for 3 days): C, 55.95; H, 8.45. Calc. for $C_{25}H_{41}O_8N.HCl.H_2O$: C, 55.84; H, 8.10%.

ACKNOWLEDGMENTS

We acknowledge our indebtedness to Mr. R. Lauzon and Dr. R. Norman Jones of these laboratories for the preparation of the infrared absorption spectra, and for discussions as to their interpretation.

REFERENCES

1. DUNSTAN, W. R. and CARR, F. H. J. Chem. Soc. **65**, 178 (1894).
2. SCHULZE, H. and LIEBNER, A. Arch. Pharm. **251**, 453 (1913); **254**, 567 (1916).

3. SCHNEIDER, W. Ber. **89**, 768 (1956).
4. SHIMA, T. and AMIYA, T. Bull. Chem. Soc. Japan, **31**, 657 (1958).
5. ROBERTS, G., GALLAGHER, B. S., and JONES, R. N. Infrared absorption spectra of steroids. Vol. 2. Interscience Publishers, Inc., New York. 1958. p. 4.
6. WARNHOFF, E. W. and WILDMAN, W. C. Chem. & Ind. 1293 (1958).
7. PRZYBYLSKA, M. and MARION, L. Can. J. Chem. **37**, 1116 (1959).
8. MAYER, H. and MARION, L. Can. J. Chem. **37**, 856 (1959).
9. WIESNER, K., BICKELHAUPT, F., and VALENTA, Z. Tetrahedron, **4**, 418 (1958).
10. JACOBS, W. A. and PELLETIER, S. W. J. Am. Chem. Soc. **78**, 3542 (1956).

ISOTOPIC AND HOT RADICAL EFFECTS IN THE REACTION OF HYDROGEN ATOMS WITH ETHYLENE[1]

A. H. TURNER[2] AND R. J. CVETANOVIĆ

ABSTRACT

Reactions of D atoms with C_2H_4, H atoms with C_2D_4, and H atoms with C_2H_4 at room temperature are compared. Pronounced differences in the extent of isotopic exchange have been found. The observed isotopic and pressure effects provide evidence for the importance of "hot" ethyl radicals in these reactions and their responsibility for isotopic exchange. The atoms are generated by the mercury-photosensitized decomposition of hydrogen and deuterium and their concentration is sufficiently small so that "atomic cracking" does not occur.

INTRODUCTION

Addition of a hydrogen atom to a molecule of ethylene leads to the formation of an energy-rich ethyl radical. The need to take into account the formation and subsequent reactions of such "hot" ethyl radicals in order to explain and reconcile seemingly divergent experimental information from various sources has been recently stressed by Bradley, Melville, and Robb (1).

In the present work additions of H and D atoms, respectively, to ethylene have been compared at various pressures. Similar systems have been recently studied by Toby and Schiff (2) but at very low pressures and at high atom-to-ethylene ratios so that "atomic cracking" was favored and methane was the main product, with only smaller amounts of ethane and ethylene and traces of butane and propane. In contrast to this in the present work the ratio of concentrations of the atoms and ethylene has been sufficiently small so that atom–radical reactions have been negligible and the only observed products are butane, ethane, and ethylene, resulting from combination and disproportionation of ethyl radicals.

From the observed effect of pressure on the amounts and isotopic structure of products, direct evidence has been obtained for the important role of "hot" ethyl radicals in these reactions as well as for their responsibility for the occurrence of isotopic exchange. The reactions have been carried out at room temperature where attack of thermalized free radicals on H_2 or D_2 is too slow to be of importance. In view of the difference in the strengths of CD and CH bonds an isotopic effect has been anticipated, and it has been found that isotopic exchange occurs when D atoms add to C_2H_4 but not when H atoms add to C_2D_4. As a result the *course* of the reaction in the two cases is quite different. Some information on the mechanism of disproportionation of ethyl radicals has also been obtained.

EXPERIMENTAL

A conventional high vacuum apparatus was used with a cylindrical quartz reaction cell 5 cm in diameter and 10 cm long. Mercury resonance radiation was obtained from a low pressure mercury arc situated some 20 cm from the reaction cell and was roughly collimated with a highly polished aluminum cylinder.

Research grade ethylene was employed and hydrogen was purified by passage through a heated palladium thimble. Tetradeuteroethylene, at least 99 atom % pure, was kindly supplied by Dr. L. C. Leitch of these laboratories.

[1]*Manuscript received February 27, 1959.*
Contribution from the Division of Applied Chemistry, National Research Council, Ottawa, Canada.
Issued as N.R.C. No. 5187.

[2]*National Research Council Postdoctorate Fellow 1955–57. Present address: Carrington Research Laboratory, Petro-Chemicals Limited, Urmston, Manchester, England.*

RESULTS

All experiments were performed at room temperature and the results for $H + C_2D_4$, $D + C_2H_4$, and $H + C_2H_4$ are given in Tables I, II, and III, respectively. Large excess of hydrogen (or deuterium) and very small amounts of ethylene were used. This ensured essentially quantitative quenching of the Hg $6(^3P_1)$ atoms by hydrogen and also made feasible mass spectrometric analyses of the recovered C_2 hydrocarbons without necessitating excessively long exposures.

Butanes and C_2 hydrocarbons were condensed in liquid nitrogen and, after the unreacted excess hydrogen was pumped off, were separated by a LeRoy still (3), measured with a constant volume gas burette, and then analyzed by mass spectrometry. No products other than those shown in the tables were detected. The observed mass balance was usually slightly below 100%, in all probability due to the difficulty of quantitatively recovering C_2 hydrocarbons from the large excess of hydrogen employed. For this reason, besides the values actually observed for ethylene and ethane, the "corrected" values are also given in the Tables I–III. The latter were calculated by assuming complete recovery of butane and scaling up the C_2 fraction to obtain 100% carbon atom balance. The amount of the C_2 fraction was, therefore, assumed to be equal to the original ethylene less twice the amount of the recovered butane. The relative proportions of ethylenes and ethanes were then calculated from the mass spectrometric analyses of the recovered C_2 fraction. In general, these corrections were not particularly large.

The mass spectrometric analyses were subject to the usual uncertainties in the values of the lighter constituents, especially when they were formed in very small amounts, because of cumulative errors in the corrections for the fragment peaks of the heavier constituents present.[3] Thus in Table I, in addition to the two ethanes, C_2D_5H and $C_2D_4H_2$,

TABLE I

Products of the reaction $H + C_2D_4$ ($25 \pm 1°$ C; exposure 60 minutes; "corrected" values are in italics)

Run	P_{H_2}, mm	C_2D_4 Taken, μmoles	Products (micromoles)					$\frac{(C_2D_5H + C_2D_4H_2)}{(C_4D_8H_2)}$	C_2D_4 Consumed, μmoles
			C_2D_4	C_2D_3H	C_2D_5H	$C_2D_4H_2$	$C_4D_8H_2$		
Q3	51.3	87.0	56.92	0.69	0.40	1.40	12.0	0.150	
			60.3	*0.73*	*0.42*	*1.48*		*0.158*	*26.7*
Q2	99.5	87.0	55.68	0.68	0.41	1.23	13.0	0.126	
			58.1	*0.71*	*0.43*	*1.28*		*0.132*	*28.9*
Q1	299.7	87.3	56.99	0.63	0.44	0.88	13.6	0.097	
			57.7	*0.64*	*0.45*	*0.89*		*0.099*	*29.6*

some $C_2D_3H_3$ is also indicated. However, the analytical uncertainty for this compound is so high that it is not certain whether it is formed at all and it has, therefore, been omitted from the table. Similarly, the figures for C_2D_3H are only approximate. The ratio of C_2D_5H and $C_2D_4H_2$, on the other hand, should be reliable. The C_4 fraction consists in this case of essentially pure $C_4D_8H_2$ (with less than 0.3% of d_9 and d_{10} butanes) and the indications are that the two H atoms are on the terminal carbon atoms. In Table II the figures for C_2H_4, C_2H_3D, C_2H_6, and C_2H_5D are probably not too accurate and, similarly, in the C_4 fraction the figures for C_4H_{10} are not too reliable.

[3]*The mass spectra of the deutero compounds from the American Petroleum Institute catalog of mass spectral data were used where available. In other cases estimates of probable fragment intensities were made by analogy with the known spectra.*

TABLE II

Products of the reaction D + C_2H_4 (25±1° C; exposure 60 minutes in $M_{8,9,10}$, 120 minutes in $M_{6,7}$; initial C_2H_4 88.5 micromoles in all experiments; "corrected" values are in italics)

Run	P_{D_2}, mm	Products (micromoles)											C_2H_4 Consumed, μmoles
		C_2H_4	C_2H_3D	C_2H_6	C_2H_5D	$C_2H_4D_2$	$C_2H_3D_3$	C_4H_{10}	C_4H_9D	$C_4H_8D_2$	$C_4H_7D_3$	Total butane	
M_{10}	49.3	51.7	4.61	4.23	1.81	—	—	0.82	3.28	4.55	0.52	9.17	
		58.3	*5.20*	*4.78*	*2.04*	—	—						*30.2*
M_9	99	36.9	5.83	4.37	1.46	—	—	0.67	3.17	5.29	0.43	9.56	
		52.7	*8.35*	*6.25*	*2.09*	—	—						*35.8*
M_8	300	55.4	3.07	4.10	1.71	—	—	0.33	1.78	8.07	0.33	10.51	
		58.3	*3.23*	*4.31*	*1.80*	—	—						*30.2*
M_6	300	23.9	2.36	4.55	2.98	1.0	0.06	0.82	4.30	17.1	1.73	23.95	
		27.7	*2.73*	*5.25*	*3.43*	*1.4*	*0.07*						*60.8*
M_7	606	21.7	1.76	2.52	2.23	1.12	—	0.95	3.33	20.25	1.05	25.58	
		27.4	*2.23*	*3.19*	*2.82*	*1.42*	—						*61.1*

The analytical uncertainties preclude a detailed quantitative analysis of the pressure dependence of the investigated reactions and for this reason no attempt has been made at present to accumulate more extensive experimental data. Fortunately, the values for the major products formed are in general reasonably reliable. There can, as well, be little doubt of the reality of the general trends observed and in particular of the pronounced difference in the complexity of the products shown in Tables I and II, respectively. As a result a number of conclusions can be safely drawn.

DISCUSSION

A significant result of the present work is the finding that isotopic exchange takes place readily when D atoms add to C_2H_4, even at relatively high pressures of D_2, while there is no exchange when H atoms add to C_2D_4. In the former case the formation of different isotopic butanes indicates, besides C_2H_4D radicals, the presence of C_2H_5 and $C_2H_3D_2$ radicals as well. In the latter case, on the other hand, only C_2D_4H radicals are present.

The amount of isotopic exchange in the reaction of D atoms with C_2H_4 (Table II) increases at lower pressures of D_2 and the following reactions have, therefore, to be considered:

$$C_2H_4 + D \rightarrow C_2H_4D^* \qquad [1]$$

$$C_2H_4D^* \rightarrow C_2H_3D + H \qquad [2]$$

$$\left[\text{or} \quad C_2H_4D^* + C_2H_4 \rightarrow C_2H_3D + C_2H_5^* \qquad [2a]\right]$$

and

$$C_2H_4D^* + M \rightarrow C_2H_4D + M \qquad [3]$$

with subsequent reactions of the H atoms produced in reaction [2] and recombination and disproportionation of the ethyl radicals formed. Addition of H atoms to the original ethylene, C_2H_4, produces C_2H_5 and as the conversion increases, D atoms will add to C_2H_3D formed in reaction [2] to give increasing amounts of $C_2H_3D_2$. The observed trends with pressure and over-all conversion in the amounts of various butanes formed in the reaction of D atoms with C_2H_4, as shown in Table II, are in qualitative agreement with the indicated reactions.

In the reaction of H atoms with C_2D_4 (Table I) the only equivalent of reaction [2]

which occurs is

$$C_2D_4H^* \rightarrow C_2D_4 + H \qquad [4]$$

$$\left[\text{or} \quad C_2D_4H^* + C_2D_4 \rightarrow C_2D_4 + C_2D_4H^* \qquad [4a]\right]$$

but not

$$C_2D_4H^* \rightarrow C_2D_3H + D \qquad [5]$$

$$\left[\text{nor} \quad C_2D_4H^* + C_2D_4 \rightarrow C_2D_3H + C_2D_5^*. \qquad [5a]\right]$$

No isotopic exchange is therefore observed.

The difference between the two cases is readily understandable in view of the difference in the bond dissociation energies between a C–D and a C–H bond as a result of the difference in the zero point energies. When a H atom adds to C_2D_4, the hot $C_2D_4H^*$ radical possesses enough excess energy to undergo reaction [4] but not reaction [5]. When a D atom adds to C_2H_4, on the other hand, the hot $C_2H_4D^*$ radical formed possesses enough excess energy to split off a D or a H atom, and both the statistical and the energetic conditions favor the latter (reaction [2]).

The isotopic structure of the ethanes formed and the pressure effects observed in these experiments are of interest from the point of view of "disproportionation" of ethyl radicals. Under conditions of the present work, ethane could not have been formed by combination of methyl radicals because if this were so appreciable quantities of propane would have been formed as well and this was not the case. Reaction of H atoms with C_2D_4 is relatively simple and the analytical results in Table I for the two ethanes formed, C_2D_5H and $C_2D_4H_2$, are reasonably reliable. (Some $C_2H_3D_3$ might have been produced as well but this is highly uncertain.) C_2D_6 is not formed. These results are not inconsistent with the "head-to-tail" disproportionation mechanism of Wijnen and Steacie (4). The ratio $C_2D_4H_2/C_4D_8H_2$ increases at lower pressures while the ratio of $C_2D_5H/C_4D_8H_2$ is pressure-independent. The following reactions ought then to be considered:

$$C_2D_4H + C_2D_4H \rightarrow C_2D_5H + C_2D_3H \qquad [6]$$

$$C_2D_4H + C_2D_4H \rightarrow C_2D_4H_2 + C_2D_4 \qquad [7]$$

$$C_2D_4H^* + C_2D_4H \rightarrow C_2D_4 + C_2D_4H_2^* \qquad [8]$$

$$C_2D_4H_2^* + M \rightarrow C_2D_4H_2^* + M. \qquad [9]$$

Reactions [6] and [7] involve thermalized radicals and lead, therefore, to a pressure-independent ratio of $C_2D_5H/C_4D_8H_2$. Reaction [8] explains the increase in $C_2D_4H_2$ as the pressure is lowered. It appears therefore necessary to assume that under the conditions employed in these experiments an H atom is abstracted much more readily than a D atom from a hot C_2D_4H radical in spite of the more favorable statistical ratio of D atoms. It is unlikely that $C_2D_4H_2$ was formed by addition of H atoms to C_2D_4H since, if anything, the pressure dependence would then be opposite to that observed.

In contrast to the results obtained in the present work, Dills and Rabinovitch (5) obtained a more complex isotopic structure of the products in an experiment with C_2D_4 and H atoms at a total pressure of about 4 mm, using a simulated point source (nozzle) of H atoms, and maintaining a spherical diffusion reaction zone. The butanes consisted of $C_4D_8H_2$ (83%), C_4D_9H (15%), and C_4D_{10} (2%) and the ethanes of C_2D_6 (63%), C_2D_5H (18%), and $C_2D_4H_2$ (19%). This was interpreted to show that the mechanism of disproportionation is not "head-to-tail" but rather that the disproportionation takes place with more than one atom migrating. This conclusion cannot be of general validity since C_2D_6, C_4D_9H, and C_4D_{10} are not formed in the same reaction under conditions employed in the present work in which the products formed are consistent with a "head-

to-tail" disproportionation. The more complex isotopic structure of the butane and ethane obtained by Dills and Rabinovitch appears to be due to the particular conditions employed by these authors and the reason for the greater complexity is not quite clear. Perhaps at very low pressures some exchange involving transfer of D atoms does occur. Since reaction [5] is slightly endothermic while [5*a*] is exothermic, the latter would perhaps provide the more readily acceptable mechanism of such an exchange. However, further experimental information is necessary before any firm conclusions can be drawn.

In the reaction of D atoms with C_2H_4 (Table II) no $C_2H_4D_2$ is formed at low conversions, and the disproportionation of C_2H_4D radicals (including also the reactions involving $C_2H_4D^*$) is, therefore, entirely by abstraction of a hydrogen atom.

$$C_2H_4D + C_2H_4D \rightarrow C_2H_5D + C_2H_3D \quad [10]$$

$$C_2H_4D + C_2H_4D^* \rightarrow C_2H_5D^* + C_2H_3D \quad [11]$$

$$C_2H_5D^* + M \rightarrow C_2H_5D + M \quad [12]$$

Abstraction of hydrogen atoms is here favored both statistically and energetically. Only at higher conversions $C_2H_4D_2$ is formed as a result of the eventual addition of D atoms to C_2H_3D produced in the course of the reaction. Formation of C_2H_6 is a consequence of the readily occurring isotopic exchange (reaction [2] or [2*a*]).[4]

A striking feature of the results of the performed experiments is the fact that the isotopic exchange (reactions such as [2] or [2*a*]) and the "hot radical" effects in the disproportionation (reactions such as [8] and [11]) persist to quite high H_2 or D_2 pressures. The occurrence of reactions such as [2] at high pressures could be explained by assuming very short natural lifetimes of the energy-rich radicals and perhaps, as is frequently done, a very readily occurring deactivation by collision for this particular process. The occurrence of reactions such as [8] and [11], on the other hand, must mean that the energy-rich ethyl radicals are deactivated in collisions only extremely slowly and are capable of undergoing preferential disproportionation (and exhibiting as well a strong isotopic effect in disproportionation), even after undergoing numerous collisions in the gas phase with H_2 or D_2 and with ethylene. Thus, for example, although in the reaction of H atoms and C_2D_4 the ratio of the concentrations of hydrogen at a pressure of 100 mm and C_2D_4H (calculated approximately from the rate of formation of $C_4D_8H_2$) is of the order of 10^5 to 10^6, there are still pronounced isotopic and pressure effects in the formation of ethane. Similarly, the pressure dependence of the ratio of disproportionation to combination in the reaction of H atoms with C_2H_4 (Table III) persists to quite high hydrogen pressures.

TABLE III

Products of the reaction H + C_2H_4 (25±1° C; exposure 60 minutes; "corrected" values are in italics)

Run	P_{H_2}, mm	C_2H_4 Taken, μmoles	Products (micromoles)			C_2H_4 Consumed, μmoles	C_2H_6/C_4H_{10}
			C_2H_4	C_2H_6	C_4H_{10}		
M_5	101	88.0	53.4	3.44	12.6		
			59.5	*3.77*		*28.5*	*0.30*
M_1	300	88.9	46.6	2.28	15.0		
			56.7	*2.79*		*32.2*	*0.19*
M_4	616	87.8	51.3	2.36	16.6		
			52.9	*2.40*		*34.9*	*0.14*

[4] *Since all ethane results from disproportionation of ethyl radicals, the relative abundance of* C_2H_6 *and* C_2H_5D *depends on the relative importance of reactions* [2] (*or* [2*a*]) *and* [3], *respectively. In the limiting case when reaction* [3] *is negligible, i.e. as pressure tends to zero, all the ethane would consist of* C_2H_6 (*as long as the conversion is small, i.e. until appreciable quantities of* C_2H_3D *are accumulated*).

In principle, it might appear to be possible to obtain quantitative information on the lifetimes of the "hot" radicals from a study of the pressure dependence of the yields of the products. The somewhat limited experimental information on pressure effects obtained in the present work and the limitations in analytical accuracy preclude a detailed analysis of this kind. However, the data in Tables I–III appear not to be consistent with the postulates of single hot radical species the rate of deactivation of which depends linearly on pressure.

The finding that abstraction of a hydrogen atom from a hot ethyl radical takes place very readily and that when the hot radical contains both H and D atoms it is the former which are abstracted, makes it necessary, by analogy, to consider the reactions of the type [2*a*] and [4*a*] as the ones which may perhaps be responsible for the isotopic exchange. However, on the basis of the available information the two types of processes (reactions [2] and [4] as contrasted with [2*a*] and [4*a*]) cannot be differentiated. Whether reactions of the latter type occur or not should perhaps be possible to establish by producing hot C_2H_5 radicals from photolysis of mercury diethyl in the presence of C_2D_4.

The ambiguity between these two types of processes may be rather general for exchange reactions of similar kind. Thus, the isotopic exchange and deuterium atom enrichment in the methane formed in the reaction of ethylene (2) or ethane (6), under conditions where atomic cracking takes place, may be due to the reactions of the type

$$CH_3 + D \rightarrow CH_3D^* \qquad [13]$$

$$CH_3D^* \rightarrow CH_2D + H \qquad [14]$$

$$CH_3D^* + M \rightarrow CH_3D + M \qquad [15]$$

as is generally assumed (6), or instead of [14]

$$CH_3D^* + R \rightarrow CH_2D + RH^*, \qquad [14a]$$

where R is D, an ethyl or a methyl radical and RH* may still be to some extent "hot", since the excess energy would be expected to be predominantly transferred into the product (RH*) in which a bond is formed. (Energetically the process simulates to a large extent the sequence of reaction [14] followed by combination of H and R to give RH*.)

Recently Lavrovskaia *et al.* (7) have proposed the following reaction as the one responsible for isotopic exchange in free radical reactions of hydrocarbons:

$$RCH_2\cdot + D_2 \rightarrow RCHD\cdot + HD. \qquad [16]$$

However, we find[5] no isotopic exchange at room temperature between thermalized ethyl radicals (from photolysis of diethyl ketone) and D_2 or C_2D_4. Reactions of this type, therefore, do not occur under conditions of the present experiments and are in general not likely to be important when thermalized radicals are involved.[6] Whether such reactions may occur when the free radicals are "hot" cannot be said at present. In such a case reaction [16] would become analogous to reaction [2*a*], tentatively suggested as a possibility in the present work.

ACKNOWLEDGMENT

The authors are grateful to Dr. A. W. Tickner for mass spectrometer analyses and their interpretation.

[5]*The authors are grateful to Mr. S. Sato for performing these experiments.*
[6]*Reaction [16] is also discussed in reference (6).*

REFERENCES

1. BRADLEY, J. N., MELVILLE, H. W., and ROBB, J. C. Proc. Roy. Soc. A, **236**, 318 (1956).
2. TOBY, S. and SCHIFF, H. I. Can. J. Chem. **34**, 1061 (1956).
3. LEROY, D. J. Can. J. Research, B, **28**, 492 (1950).
4. WIJNEN, M. H. J. and STEACIE, E. W. R. Can. J. Chem. **29**, 1092 (1951).
5. DILLS, D. H. Lifetimes for decomposition of vibrationally excited dideuteroethyl radicals. Ph.D. Thesis, University of Washington. 1956.
6. BERISFORD, R. and LEROY, D. J. Can. J. Chem. **36**, 983 (1958).
7. LAVROVSKAIA, G. K., MARDALEISHVILI, R. E., and VOEVODSKII, V. V. Voprosy Khim. Kinetiki, Kataliza, i Reakstsionnoi Sposobnosti, Akad. Nauk S.S.S.R., Otdel. Khim. Nauk, 40 (TT-701) (1955).

THE REACTION OF POTASSIUM PERSULPHATE WITH THIOGLYCOLIC ACID IN AQUEOUS SOLUTION[1]

J. F. HENDERSON[2] AND C. A. WINKLER

ABSTRACT

The oxidation of thioglycolic acid to dithiodiglycolic acid by potassium persulphate in aqueous solution in the absence of iron did not yield reproducible results, but satisfactory data were obtained when ferrous or ferric ions were added to the system. The catalyst cycle appears to be that ferrous ion is oxidized by persulphate to ferric ion, and the ferric ion is reduced back to ferrous ion by thioglycolic acid. In the absence of persulphate, the reaction of ferric ion with thioglycolic acid in acid solution (pH <3) was second order in both ferric ion and thioglycolic acid concentrations and was markedly inhibited by hydrogen ion.

INTRODUCTION

The rate-controlling step in the thermal decomposition of persulphate ion in aqueous solution has been postulated to be the first-order homolytic dissociation of persulphate to yield sulphate ion radicals (1, 2, 3).

$$S_2O_8^{=} \rightarrow 2SO_4^{\cdot -}$$

The addition of organic compounds to solutions of persulphate in general increases the rate of decomposition of the persulphate ion, but the mechanisms of many of these reactions are not well understood (2, 4, 5, 6, 7).

The reactions of potassium persulphate with alkyl mercaptans in glacial acetic acid – water solution have been discussed in a previous paper from this laboratory (4). Secondary salt effects were observed which tended to make interpretation of the results difficult, but it seemed that persulphate decomposed unimolecularly to yield two sulphate ion radicals which either reacted with the mercaptan or recombined in a solvent "cage".

For the decomposition of persulphate ion in the presence of certain other organic compounds, e.g. methanol and hydrazobenzene, no evidence for the existence of a solvent cage has been found (2, 5, 6). Furthermore, it has been postulated that the sulphate ion radicals in these reaction mixtures were produced by direct reaction between persulphate ion and the organic compound rather than by unimolecular decomposition of persulphate ion (2, 5), though for the persulphate–methanol reaction there is now doubt that this view is correct (6).

The present investigation was planned originally with the intention of obtaining more information about the reactions of persulphate ion with organic compounds, particularly those containing the sulphydryl group. Thioglycolic acid was chosen to permit experiments in aqueous solution, hence avoiding the difficulties encountered in the earlier investigation (4).

Preliminary experiments (8), which will not be described, indicated that 2 moles of thioglycolic acid were oxidized by 1 mole of persulphate to yield 1 mole of dithiodiglycolic acid, but measurements of the reaction rate were quite erratic. Reproducible data were obtained, however, in the presence of soluble iron salts as catalyst, and it was decided to investigate this catalyzed reaction.

[1]*Manuscript received February 5, 1959.*
Contribution from the Physical Chemistry Laboratory, McGill University, with financial assistance from the Canadian Kodak Co., Ltd., and the National Research Council.
[2]*Holder of a Canadian Kodak Company, Ltd., Fellowship.*

EXPERIMENTAL

Fisher "purified" thioglycolic acid was distilled rapidly at 8 mm Hg and 95° C, and the distillate was stored at 0° C under an atmosphere of nitrogen (9). Its purity was estimated to be 99.5% by diluting weighed quantities with water and titrating these with standard iodine solution, using starch as the indicator.

The other materials employed in this investigation, which were supplied by Brickman and Company, Montreal, were of reagent grade and were used as received.

Either ferrous or ferric ammonium sulphate was used to catalyze the reaction. The ionic strength of the reaction mixture was adjusted by adding potassium sulphate, sulphuric acid, or mixtures of the two. In calculating the ionic strength, allowance was made for the incomplete dissociation of KSO_4^- and HSO_4^- ions (10, 11).

Both the standardized solutions of the reactants and the reaction mixtures were prepared with laboratory distilled water. Prior to mixing, the reactant solutions were swept with nitrogen (99.9%) which was saturated with water vapor and used without further purification. The reaction mixtures were swept with moist nitrogen while experiments were in progress at 24.8±0.02° C.

The progress of the reaction was determined by transferring aliquots from the reaction mixture to 25 ml of approximately 20% phosphoric acid and estimating the thioglycolic acid content by titration with a standard solution of iodine in a solution of potassium iodide. The phosphoric acid, by complexing the ferric ion, served to slow the reaction and to prevent possible complications in the subsequent titration with iodine. Reaction of persulphate with iodide ion during the iodimetric titration was found to be negligible.

For experiments in 0.15 *M* sulphuric acid, both the progress of reaction and the concentration of ferric ion were determined by periodically removing aliquots from the reaction mixture and recording their ultraviolet spectra for wavelengths up to 400 mμ. From the data in Table I it may be inferred that appreciable complex formation did not occur between any of the components present in the reaction mixtures. The concentration of ferric ion in reaction mixtures was estimated from the optical density measured at 325 mμ. The optical densities at lower wavelengths were then corrected for the contri-

TABLE I

Optical density of aqueous solutions of the components and of various mixtures of the components present in the iron-catalyzed persulphate - thioglycolic acid reaction mixtures (sulphuric acid 0.15 M/l. (mole/liter))

Composition of solution	Optical density						
	D330	D310	D290	D270	D250	D230	D210
A. Pure components							
8.02×10^{-4} *M* $S_2O_8^=$				0.005	0.026	0.089	0.367
0.955×10^{-3} *M* TSH				0.005	0.088	0.250	0.722
1.00×10^{-3} *M* TSH				0.005	0.075	0.240	0.783
1.91×10^{-3} *M* TSH				0.012	0.162	0.479	
1.00×10^{-4} *M* TSST				0.024	0.098	0.189	0.331
4.00×10^{-4} *M* Fe^{++}					0.010	0.018	0.020
4.45×10^{-5} *M* Fe^{+++}	0.069	0.110	0.102	0.087	0.157	0.235	0.230
8.00×10^{-5} *M* Fe^{+++}	0.134	0.199	0.185	0.160	0.276	0.408	0.415
B. Mixtures of components							
8.02×10^{-4} *M* $S_2O_8^=$ and 1.91×10^{-3} *M* TSH				0.022	0.190	0.572	
8.02×10^{-4} *M* $S_2O_8^=$ and 4.45×10^{-5} *M* Fe^{+++}	0.069	0.113	0.109	0.098	0.185	0.330	0.630
4.00×10^{-4} *M* Fe^{+++} and 0.955×10^{-3} *M* TSH				0.005	0.103	0.272	0.754
1.00×10^{-4} *M* TSST and 1.00×10^{-3} *M* TSH				0.035	0.184	0.450	
1.00×10^{-4} *M* TSST and 4.00×10^{-4} *M* Fe^{++}				0.028	0.118	0.219	0.364
1.00×10^{-4} *M* TSST and 8.00×10^{-5} *M* Fe^{+++}	0.134	0.199	0.209	0.185	0.386	0.830	0.827

bution by ferric ion and, by using this corrected value, the concentrations of the remaining components in the reaction mixture at any time were calculated using the following equation,

$$[\mathrm{TSST}] = \frac{D - \epsilon_{\mathrm{TSH}}[\mathrm{TSH}]_0 - \epsilon_{S_2O_8^=}[S_2O_8^=]_0}{\epsilon_{\mathrm{TSST}} - 2\epsilon_{\mathrm{TSH}} - \epsilon_{S_2O_8^=}}$$

where D represents the corrected optical density, ϵ the respective extinction coefficients, and $[\mathrm{TSH}]_0$ and $[S_2O_8]_0$ the initial concentrations of thioglycolic acid and persulphate respectively. The results obtained using the spectrophotometric method were in excellent agreement with those obtained using the volumetric method (Fig. 4).

RESULTS

Experiments at a pH of approximately 2 showed the reaction to be first order in the persulphate concentration and zero order in the thioglycolic acid concentration. The first-order rate constant for persulphate disappearance, k_1' in Table II, was directly proportional to the concentration of iron in the system, which indicates that, in the presence of added iron, direct reaction between persulphate and thioglycolic acid was negligible. The effect of ionic strength on the calculated second-order rate constant, $k_1 = k_1'/[\mathrm{Fe}]$, is shown in Fig. 1, and it is apparent that the rate-controlling step of the reaction consists of interaction between doubly charged ions of the opposite sign.

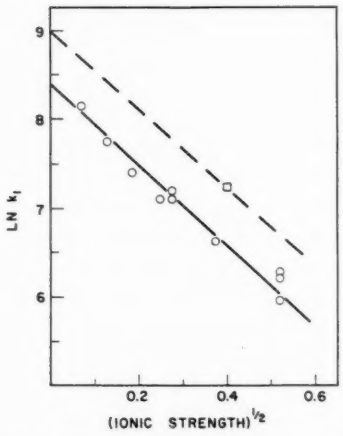


FIG. 1. Variation of the second-order rate constant, k_1, with the ionic strength: —□— see ref. (16, 17).

For reaction mixtures in which the concentration of sulphuric acid was 0.15 M and in which the iron was initially in the ferrous state, the initial rates of reaction and the first-order rate constants at zero time were functions of the initial concentrations of both the persulphate and the thioglycolic acid (Table III). Furthermore, the concentration of ferric ion reached a stationary value which increased with increasing persulphate concentration and decreased with increasing thioglycolic acid concentration (Figs. 2 and 3). Division of the rate of disappearance of persulphate in these reaction mixtures by the product of the persulphate and ferrous ion concentrations at a given instant of time yielded a second-order rate constant corresponding to k_1 which was independent of the reaction mixtures, and for which the average value (651 l. mole^{-1} min^{-1}) was in reasonable agreement with that shown in Fig. 1 at the same ionic strength. As observed

TABLE II

The effect of different initial concentrations of ferric ion, persulphate, and thioglycolic acid on the rate of disappearance of persulphate at pH $\simeq$ 2

Initial $[Fe^{+++}]$ ($\times 10^6$)	Initial $[S_2O_8^{=}]$ ($\times 10^3$)	Initial [TSH] ($\times 10^3$)	$[K_2SO_4]$	$[H_2SO_4]$	Ionic strength (μ)	k_1' (min^{-1})	$k_1 = k_1'/[Fe]$ ($l.\ M^{-1}\ min^{-1}$)
10.0	1.60	4.50	0.00	0.10	0.269	0.0492	492
10.0	3.20	9.00	0.00	0.10	0.269	0.0492	497
10.0	6.40	13.50	0.00	0.10	0.269	0.0492	460
10.0	6.40	4.50	0.00	0.10	0.269	0.0380	380
4.0	3.19	9.72	0.0057	0.010	0.038	0.066	1650
4.0	3.19	4.86	0.0057	0.010	0.038	0.066	1650
4.0	3.19	14.94	0.0057	0.010	0.038	0.066	1650
2.0	8.00	9.40	0.0095	0.10	0.272	0.00782	391
2.0	4.00	9.40	0.0095	0.10	0.272	0.00794	397
2.0	2.40	9.40	0.0095	0.10	0.272	0.00782	391
2.0	1.60	9.40	0.0095	0.10	0.272	0.00794	397
0.8	4.00	9.35	0.0095	0.10	0.272	0.00329	410
4.0	4.00	9.35	0.0095	0.10	0.272	0.01640	410
10.0	4.00	9.35	0.0095	0.10	0.272	0.0398	398

TABLE III

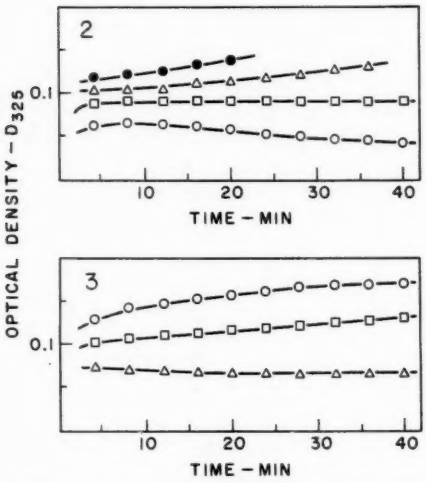
The effect of different initial concentrations of ferrous ion, persulphate, and thioglycolic acid on the rate of disappearance of persulphate in 0.15 *M* sulphuric acid (ionic strength = 0.224)

Initial $[Fe^{++}]$ ($\times 10^5$)	Initial $[S_2O_8^{=}]$ ($\times 10^3$)	Initial [TSH] ($\times 10^3$)	Initial $\frac{d[S_2O_8^{=}]}{dt}$ ($M\ l.^{-1}\ min^{-1} \times 10^5$)	Initial k_1' (min^{-1})
10.0	2.00	10.95	7.1	0.0368
10.0	4.00	10.95	15.5	0.0359
10.0	6.00	10.95	20.0	0.0320
10.0	8.00	10.95	24.0	0.0299
10.0	2.00	4.38	7.0	0.0347
10.0	4.00	4.38	12.7	0.0304
10.0	8.00	4.38	20.0	0.0253
10.0	4.00	2.18	12.1	0.0285
10.0	4.00	7.63	13.0	0.0331
10.0	4.00	15.30	13.0	0.0370
5.0	4.00	10.95	7.5	0.0161
15.0	4.00	10.95	22.0	0.0630

previously for pH$\sim$2, the initial rate of reaction extrapolated to zero when the initial concentration of ferrous ion was decreased to zero indicating that direct reaction between persulphate ion and thioglycolic acid was negligible.

When the added iron was initially in the ferric state, the reaction was preceded by an induction period, but following this induction the rate of reaction was the same as for experiments in which the iron was initially in the ferrous state (Fig. 4). The stationary-state concentration of ferric ion was independent of the valence state of the iron at zero time, although it was not attained for several minutes when the iron was initially in the ferric state. The rate of disappearance of ferric ion during the greater part of the induction period, i.e. before its concentration reached a stationary value, was not influenced by the presence of persulphate (Fig. 5).

To study the reaction between ferric ion and thioglycolic acid in sulphuric acid solutions, the disappearance of ferric ion was followed by the change in optical density at 305 mμ. The thioglycolic acid concentration was in sufficient excess over that of ferric ion to be considered independent of time. The slopes of the straight lines in Fig. 6, which show the second-order dependence of the reaction rate on the ferric ion concentration, are



FIGS. 2 AND 3. Effect of different initial concentrations of persulphate and of thioglycolic acid on the optical density at 325 mμ. Initial concn. Fe^{++} 10.0×10^{-5} M/l. Concn. H_2SO_4 0.15 M/l.

FIG. 2. Initial concn. TSH
Initial concn. $S_2O_8^=$
○ 0.01095 M/l.
□ 0.00200 M/l.
△ 0.00400 M/l.
● 0.00600 M/l.
0.00800 M/l.

FIG. 3. Initial concn. $S_2O_8^=$
Initial concn. TSH
○ 0.00400 M/l.
□ 0.00218 M/l.
△ 0.00763 M/l.
0.01530 M/l.

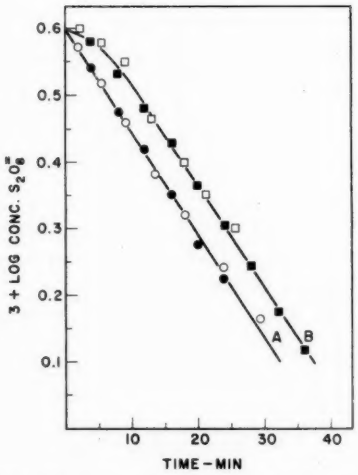


FIG. 4. Effect of the initial oxidation state of the dissolved iron on the rate of disappearance of persulphate.

A Iron initially ferrous
B Iron initially ferric
○, □ Iodimetric analysis
●, ■ Spectrophotometric analysis

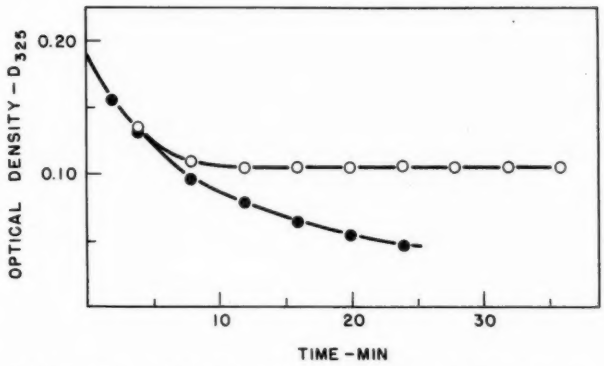


FIG. 5. The variation of optical density at 325 mμ with time when the iron was initially in the ferric state.

Initial concn. TSH 0.01085 M/l. Initial concn. $S_2O_8^=$ ○ 0.00400 M/l. ● 0.00000 M/l.

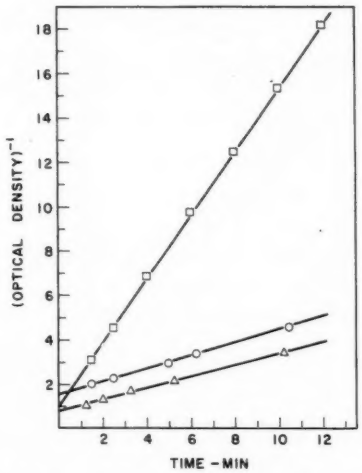


FIG. 6. Effect of different initial concentrations of thioglycolic acid on the rate of reaction between ferric ion and thioglycolic acid.

Initial concn. TSH ○ 0.00488 M/l. □ 0.01220 M/l. △ 0.00488 M/l.

proportional to the square of the thioglycolic acid concentration, indicating the over-all reaction to be of the fourth order. The effect of sulphuric acid concentration on the calculated fourth-order rate constant, k_4''', is shown in Table IV.

When the ferric ion and thioglycolic acid were mixed initially in these experiments, a blue complex was formed which faded rapidly as observed in earlier investigations (13, 14). However, extrapolation of the measured optical densities at 305 mμ to zero time indicated that the optical density of ferric ion was not influenced by thioglycolic acid, and hence that ferric ion – thioglycolic acid complexes were highly dissociated.

The dependence of the fourth-order rate constant on hydrogen ion concentration was

TABLE IV

Variation of k_4''', the calculated fourth-order rate constant for the ferric ion – thioglycolic acid reaction, with the concentration of sulphuric acid

$[H_2SO_4]$	k_4''' ($l.^2\ M^{-2}\ min^{-1} \times 10^{-6}$)
0.050	242
0.100	27.6
0.150	10.3

determined more exactly by making experiments at various levels of perchloric acid when the ionic strength of the reaction mixtures was adjusted to 1.00 with sodium perchlorate. The reaction was followed by periodically removing aliquots of the reaction mixture and estimating the ferric ion content colorimetrically as the thiocyanate complex (12). Again the thioglycolic acid concentration was in sufficient excess over that of ferric ion to be effectively independent of time. The fourth-order rate constant, k_4', was found to be inversely proportional to approximately the fourth power of the hydrogen ion concentration (Fig. 7). Low concentrations of sulphate ion in these reaction mixtures also caused inhibition (Table V).

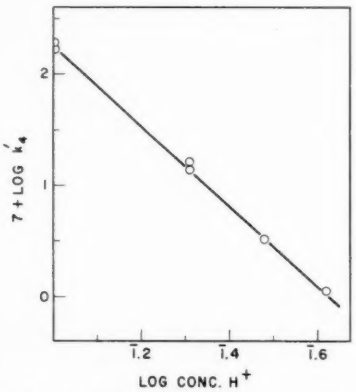


FIG. 7. Effect of perchloric acid concentration on k_4'.

TABLE V

Effect of sulphuric acid concentration on the fourth-order rate constant, k_4'' (ionic strength = 1.00)

$[HClO_4]$	$[H_2SO_4]$	$[SO_4^{=}]$	$[H^+]$	k_4'' ($l.^2\ M^{-2}\ min^{-1} \times 10^{-7}$)	k_4' ($l.^2\ M^{-2}\ min^{-1} \times 10^{-7}$)
0.101	0.0098	0.0070	0.118	63.8	110
0.101	0.0098	0.0070	0.118	71.4	110
0.101	0.0245	0.0170	0.143	22.1	52.5

NOTE: Values of k_4', which were interpolated from Fig. 7, were determined under identical experimental conditions except that sulphate ion was absent.

The fourth-order rate constant, k_4''', for the ferric ion – thioglycolic acid reaction was also determined by dividing the rate of formation of dithiodiglycolic acid in persulphate –

thioglycolic acid reaction mixtures by the product of the square of the ferric ion concentration and the square of the thioglycolic acid concentration. Its value in these reaction mixtures was approximately 100 times greater than that determined in systems which were identical in every way except that persulphate was absent.

A brief investigation of the reaction between persulphate ion and ferrous ion in the absence of thioglycolic acid showed (Table VI) that the reaction was second order, and

TABLE VI

Comparison of second-order rate constants, k_1, for the ferrous ion – persulphate reaction with those interpolated from Fig. 1

Initial $[S_2O_8^{=}]$	Initial $[Fe^{++}]$	$[K_2SO_4]$	$[H_2SO_4]$	Ionic strength (μ)	k_1 (l. M^{-1} min^{-1})	k_1* (l. M^{-1} min^{-1})
7.88	10.0	0.10	0.0098	0.272	452	448
7.88	10.0	0.00	0.098	0.159	782	730
4.00	10.0	0.00	0.15	0.224	575	513
4.00	20.0	0.00	0.15	0.224	535	513
8.00	20.0	0.00	0.15	0.224	571	513

*See Fig. 1.

that the second-order rate constants, k_1, were in good agreement with those interpolated from Fig. 1. The rate of appearance of ferric ion in these reaction mixtures was estimated from the change in optical density at 305 mμ.

DISCUSSION

Thioglycolic acid in no way influenced the rate of the persulphate ion – ferrous ion reaction, whereas the presence of persulphate caused the reaction between ferric ion and thioglycolic acid to occur more rapidly. It is apparent that the catalyst cycle for the reaction between persulphate ion and thioglycolic acid, in the presence of iron, involves persulphate oxidation of ferrous ion to ferric ion, which is then reduced by thioglycolic acid to ferrous ion.

The mechanism for the reaction between persulphate ion and ferrous ion appears to be well established (15, 16, 17).

$$S_2O_8 + Fe^{++} \rightarrow Fe^{+++} + SO_4^{-\cdot} + SO_4^{=} \quad [1]$$

$$SO_4^{-\cdot} + Fe^{++} \rightarrow Fe^{+++} + SO_4^{=} \quad [2]$$

The calculated second-order rate constant, k_1, obtained in this investigation was approximately one-half that obtained by Williams *et al.* (16, 17). The difference might result from the much larger hydrogen ion concentrations (100- to 1000-fold) in the present experiments.

For the reaction between ferric ion and thioglycolic acid the results may be explained by postulating the existence of a complex containing two ferric ions and two thioglycolic acid molecules, the reaction rate being governed by the first-order decomposition of this complex. That complexes in the ferric ion – thioglycolic acid system do exist is indicated by the blue color that was produced when the two reactants were mixed initially. Lamfrom and Nielsen (14) have shown that the blue complex disappeared by a first-order process at $-35°$ C in a solvent consisting of 6 parts by volume of ethanol to 10 parts by volume of water. It would appear, therefore, that the blue complex might well consist of two ferric ions and two thioglycolic acid molecules.

The inhibition by hydrogen and by sulphate ions suggests that mercaptide and hydrated ferric ions were the reactive species and the proposed mechanism is as follows:

$$Fe^{+++} + SO_4^{=} \rightleftarrows FeSO_4^{+}, \quad [4]$$

$$FeSO_4^{+} + SO_4^{=} \rightleftarrows Fe(SO_4)_2^{-}, \quad [5]$$

$$Fe^{+++} + ClO_4^{-} \rightleftarrows FeClO_4^{++}, \quad [6]$$

$$Fe^{+++} + H_2O \rightleftarrows FeOH^{++} + H^{+}, \quad [7]$$

$$TSH \rightleftarrows TS^{-} + H^{+}, \quad [8]$$

$$FeOH^{++} + TS^{-} \rightleftarrows FeOHTS^{+}, \quad [9]$$

$$2FeOHTS^{+} \rightleftarrows Fe_2(OH)_2(TS)_2^{++}, \quad [10]$$

$$Fe_2(OH)_2(TS)_2^{++} \rightarrow 2Fe^{++} + {}^{-}TSST^{-} + 2H_2O. \quad [11]$$

For the experiments made in the absence of sulphate ion, the following relation is obtained,

$$k_4'\,[\text{ferric}]^2\,[\text{thioglycolic acid}]^2 = \frac{k_{11}K_{10}K_9^{\,2}K_8^{\,2}K_7^{\,2}}{[H^+]^4}\,[Fe^{+++}]^2\,[TSH]^2 \quad (i)$$

where [ferric] and [thioglycolic acid] represent the total concentrations of ferric ion and thioglycolic acid respectively in the reaction mixture. Owing to the relatively large concentrations of hydrogen ion used in these experiments, the concentrations of $FeOH^{++}$ and TS^{-} ions should be small (18, 19), and since complex formation between ferric and perchlorate ions occurs only to a very limited extent (20), it follows that

$$[\text{ferric}] \simeq [Fe^{+++}]$$

and

$$[\text{thioglycolic acid}] \simeq [TSH].$$

Therefore

$$k_4' = \frac{k_{11}K_{10}K_9^{\,2}K_8^{\,2}K_7^{\,2}}{[H^+]^4}. \quad (ii)$$

In the presence of low concentrations of sulphate ion

$$[\text{ferric}] = [Fe^{+++}][1+K_4(SO_4^{=})]$$

and

$$k_4'' = \frac{k_{11}K_{10}K_9^{\,2}K_8^{\,2}K_7^{\,2}}{[H^+]^4\,[1+K_4(SO_4^{=})]^2}. \quad (iii)$$

By combining equations (ii) and (iii),

$$K_4 = \frac{\sqrt{(k_4/k_4'')}-1}{[SO_4^{=}]}. \quad (iv)$$

The value of K_4, the association constant of the ferric ion – sulphate complex, calculated in this manner was approximately one-half that determined by Whitekar and Davidson (21).

In the reaction mixtures which were 0.05 to 0.15 M in sulphuric acid, the concentration of free ferric ion may be taken as negligible (21), i.e.

$$[\text{ferric}] = [Fe^{+++}][K_4(SO_4^{=})+K_4K_5(SO_4^{=})^2]$$

and

$$k_4''' = \frac{k_{11}K_{10}K_9^{\,2}K_8^{\,2}K_7^{\,2}}{[H^+]^4\,[K_4(SO_4^{=})+K_4K_5(SO_4^{=})^2]^2}. \quad (v)$$

It is interesting to note that the mechanism for the reaction between ferric ion and thioglycolic acid in weakly acidic and in alkaline solution differs markedly from that suggested here for the reaction in more strongly acidic solution (14, 22, 23).

If no account is taken of the relatively rapid ferric ion – thioglycolic acid reaction which occurred in the presence of persulphate, the following sequence of reactions may be suggested for the iron-catalyzed reaction between persulphate and thioglycolic acid:

$$S_2O_8 + Fe^{++} \rightarrow Fe^{+++} + SO_4 + SO_4 \quad [1]$$

$$SO_4 + Fe^{++} \rightarrow Fe^{+++} + SO_4 \quad [2]$$

$$SO_4 + TSH \rightarrow TS\cdot + HSO_4 \quad [3]$$

$$Fe^{+++} + SO_4^{=} \rightleftarrows FeSO_4^{+} \quad [4]$$

$$FeSO_4^{+} + SO_4^{=} \rightleftarrows Fe(SO_4)_2^{-} \quad [5]$$

$$Fe^{+++} + H_2O \rightleftarrows FeOH^{++} + H^{+} \quad [6]$$

$$TSH \rightleftarrows TS^{-} + H^{+} \quad [7]$$

$$FeOH^{++} + TS^{-} \rightleftarrows FeOHTS^{+} \quad [8]$$

$$2FeOHTS^{+} \rightleftarrows Fe_2(OH)_2(TS)_2^{++} \quad [9]$$

$$Fe_2(OH)_2(TS)_2^{++} \rightarrow 2Fe^{++} + {}^{-}TSST^{-} + H_2O \quad [10]$$

$$2TS\cdot \rightarrow TSST \quad [11]$$

Step [1] was rate controlling in reaction mixtures when the pH was approximately 2, but the reduction of ferric ion by thioglycolic was not rapid relative to step [1] in reaction mixtures 0.15 *M* in sulphuric acid. If step [2] is assumed negligible relative to [3] because of the large excess of thioglycolic acid over ferrous ion, the steady-state assumption may be applied to the concentrations of ferric and ferrous ions to yield the following relation,

$$\frac{D_m - D}{D^2} = \frac{k_4'''}{\epsilon k_1} \frac{[TSH]^2}{[S_2O_8^{=}]} \quad \text{(vi)}$$

where D represents the optical density of the reaction mixture at 325 mμ, D_m the optical density if all the iron had been in the ferric state, and ϵ the extinction coefficient of ferric ion. In actual fact the results from the experiments made in 0.15 *M* sulphuric acid were found to fit the empirical equation,

$$\frac{D_m - D}{D^2} = \frac{k}{\epsilon k_1 [Fe]^x [TSH]_0} \frac{[TSH]^2}{[S_2O_8^{=}]} \quad \text{(vii)}$$

where $[Fe]^x$ represents the concentration of iron in the system raised to some power x and $[TSH]_0$, the initial concentration of thioglycolic acid.

Furthermore, the values of

$$\frac{k}{k_1 [Fe]^x [TSH]_0}$$

were of the order of 100 times greater than the value of k_4'''/k_1 (Table VII), owing to the influence of persulphate ion on the ferric ion – thioglycolic reaction.

Additional steps in the reaction sequence already shown are necessary to explain, not only the marked influence of persulphate ion on the over-all rate, but also the induction period when iron was initially in the ferric state, and the lack of influence by persulphate on the rate at which ferric ion concentration is decreased to its stationary value during the induction period. A tentative proposal is that either a sulphate ion radical or a

thiyl radical reacts rapidly with a ferric ion – thioglycolic acid complex,

$$SO_4^{-} + \text{Fe (III) TSH-complex} \rightarrow Fe^{++} + H^+ + \text{TSST} + HSO_4^- \quad [12]$$

$$TS\cdot + \text{Fe (III) TSH-complex} \rightarrow Fe^{++} + \text{TSST} + H^+. \quad [13]$$

TABLE VII

Comparison of the calculated value of k_4'''/k_1 with the value $\frac{k}{k_1[Fe]^x[TSH]_0}$

Initial $[TSH]_0$ ($\times 10^3$)	$\frac{k}{k_1[Fe]^x[TSH]_0}$ ($\times 10^{-4}$)	k_4'''/k_1 ($\times 10^{-4}$)
10.95	92.3	1.85
4.36	333	1.85

In solutions in which the iron is present initially as ferric ion, free radicals will not be produced at zero time, and reactions [12] and/or [13] will not be important. Due to the production of ferrous ion, however, the persulphate – ferrous ion reaction will begin to occur, giving rise to free radicals and hence to the rapid reactions [12] and/or [13]. Therefore, during the early stages of the reaction before ferric ion and in all probability the free-radical concentrations become stationary, the rate of reduction of ferric ion by thioglycolic acid will increase with time causing a corresponding increase in the over-all reaction rate, and an induction period will result. However, when the iron is initially in the ferrous state, the sulphate ion radicals and ferric ion will be produced at zero time, and reactions [12] and/or [13] will assume an important role at the beginning.

A similar mechanism has been suggested for the uptake of oxygen by mixtures of ferric ion and thioglycolic acid in mildly acidic solution (14).

With existing information, it is extremely difficult to determine either the structure of the complex which entered into this reaction or the nature of the radical, whether sulphate ion or thiyl, which reacted with the complex. Because sulphate ion radicals may react with both ferrous ion and thioglycolic acid, the situation is even more complex, since the relative rates of these two reactions are unknown.

In conclusion, it is apparent that to investigate the uncatalyzed persulphate – thioglycolic acid reaction, as was intended originally, the concentration of dissolved iron in the reaction mixtures, and in all probability the concentration of other metallic ions, would have to be very low.

REFERENCES

1. GREEN, L. and MASSON, D. J. Chem. Soc. **97**, 2083 (1910).
2. BARTLETT, P. D. and COTMAN, D. J. J. Am. Chem. Soc. **71**, 1419 (1949).
3. KOLTHOFF, I. M. and MILLER, I. K. J. Am. Chem. Soc. **73**, 3055 (1951).
4. EAGER, R. S. and WINKLER, C. A. Can. J. Research, B, **26**, 527 (1948).
5. WHALLEY, B. J. P., EVANS, H. G. V., and WINKLER, C. A. Can. J. Chem. **34**, 1154 (1956).
6. KOLTHOFF, I. M., MEEHAN, E. J., and CARR, E. M. J. Am. Chem. Soc. **75**, 1439 (1953).
7. MALINOSKI, E. R. and LEVITT, L. S. Abstracts of Papers, 132nd Meeting, American Chemical Society, New York. 1957. p. 77P.
8. HENDERSON, J. F. Ph.D. Thesis, McGill University, Montreal. 1958.
9. LEUSSING, D. L. and KOLTHOFF, I. M. J. Electrochem. Soc. **100**, 334 (1953).
10. JENKINS, I. L. and MONK, C. B. J. Am. Chem. Soc. **72**, 2695 (1950).
11. KERKER, M. J. Am. Chem. Soc. **79**, 3664 (1957).
12. KOLTHOFF, I. M. and SANDEL, E. B. Textbook of quantitative analysis. 3rd ed. The Macmillan Co., New York. 1952. p. 635.
13. CANNON, B. H. and RICHARDSON, G. W. Biochem. J. **23**, 1242 (1929).
14. LAMFROM, H. and NIELSEN, S. O. J. Am. Chem. Soc. **79**, 1966 (1957).

15. KOLTHOFF, I. M., MEDALIA, A. I., and BAAEN, H. P. J. Am. Chem. Soc. **73**, 1733 (1951).
16. FORDHAM, J. W. L. and WILLIAMS, H. L. J. Am. Chem. Soc. **73**, 4855 (1951).
17. ORR, R. J. and WILLIAMS, H. L. J. Am. Chem. Soc. **77**, 3715 (1955).
18. TURNER, R. C. and MILES, K. E. Can. J. Chem. **35**, 1002 (1957).
19. LARSSON, E. Z. anorg. u. allgem. Chem. **172**, 375 (1928).
20. SYKES, K. W. The kinetics and mechanism of inorganic reactions in solution. Chem. Soc. (London), Spec. Publ. No. 1, p. 64 (1954).
21. WHITEKAR, R. A. and DAVIDSON, N. J. Am. Chem. Soc. **75**, 3081 (1953).
22. LEUSSING, D. L. and KOLTHOFF, I. M. J. Am. Chem. Soc. **75**, 3904 (1953).
23. LEUSSING, D. L. and NEWMAN, L. J. Am. Chem. Soc. **78**, 562 (1956).

THE PURIFICATION, DETERMINATION, AND NEUTRON CAPTURE CROSS SECTION OF ACTINIUM-227[1]

M. J. CABELL[2]

ABSTRACT

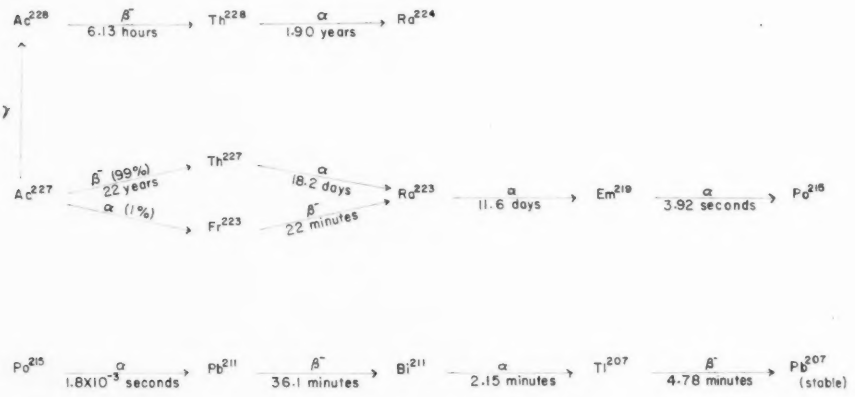
Procedures are described for (*a*) the separation by ion exchange chromatography of submicrogram quantities of Ac^{227} from its daughters and (*b*) the absolute determination of Ac^{227} by measurement of the rate of growth of α-particle emission from an initially pure source. These procedures are utilized in the determination of the reactor and thermal neutron capture cross sections and the resonance capture integral of Ac^{227} by an activation method.

Assuming 36.5 barns for the thermal neutron capture cross section of Co^{59}, a value of $\sigma_{2200} = 814 \pm 13$ barns has been obtained for Ac^{227}. The resonance capture integral (from 0.5 ev to ∞) was found to be 1177 ± 19 barns relative to 48.6 barns for the resonance capture integral of Co^{59}.

INTRODUCTION

Hughes and Schwartz (1) quote 510 ± 40 barns for the thermal neutron capture cross section of Ac^{227} as determined by a pile oscillator method and 520 ± 50 barns as determined by an activation method; no experimental data are available for either of these measurements. Timma and Russell (2), also using a pile oscillator method, obtained 493 ± 53 barns for the thermal neutron capture cross section. No measurements of differential cross sections or of the resonance capture integral of this isotope have so far been reported in the literature. This paper describes determinations of the reactor and thermal neutron capture cross sections and the resonance capture integral of Ac^{227} by an activation method.

The following nuclear reactions are involved:



The method used is similar to that described by Cabell (3) and by Cabell, Eastwood, and Campion (4) and involved the irradiation in a high flux position of the NRX reactor of submicrogram amounts of Ac^{227} (*a*) unshielded and (*b*) shielded by 0.0635 cm cadmium. Cobalt monitors were attached to measure both the thermal and the epicadmium fluxes.

[1]*Manuscript received January 23, 1959.*
Contribution from the Research Chemistry Branch, Atomic Energy of Canada Limited, Chalk River, Ontario. Issued as A.E.C.L. No. 745.
[2]*Present address: Atomic Energy Research Establishment, Harwell, Berkshire, England.*

After irradiation the actinium was purified by ion exchange and mounted on thin films for counting. The Ac^{228} content of each source was then determined from measurements of the change in its absolute β-disintegration rate with time. The rise in absolute α-disintegration rate of the same source with time gave a measure of its Ac^{227} content. From these data and the known fluxes the effective cross section for Ac^{227} for pile neutrons, its thermal capture cross section, and its resonance capture integral were calculated.

EXPERIMENTAL METHODS

(*a*) *Preparation of Pure Actinium-227*

A sample of Ac^{227} which had been only partially separated from neutron-irradiated Ra^{226} and contained large quantities of water-soluble salts as additional impurities was received from the Commercial Products Division (Ottawa) of Atomic Energy of Canada Ltd. The actinium was freed from salts by repeated coprecipitation with ferric hydroxide, the iron was removed by anion exchange, and the actinium was separated from the products of its decay and from Ra^{226} by cation exchange (see section on chemical purification). The actinium was finally obtained in 2 ml of 6 *M* nitric acid and this solution was used as a stock for all irradiations.

Aliquots taken from the stock solution were evaporated to dryness on platinum disks and the spectra of the α- and β-particles they emitted were examined. An α-pulse analysis carried out 4 hours after the final separation showed that α-particles from Ac^{227} accounted for about half the total α-particle activity. Separate and growing peaks were identified due to the α-particles from Th^{227}, Ra^{223}, Em^{219}, Po^{215}, and Bi^{211} and accounted for the remainder of the α-particle activity. No evidence of the presence of α-particles from Ra^{226} could be found.

Another source was counted in an internal proportional counter whose anode potential was adjusted for β-particle counting, and the counting rate of the source was determined both uncovered and covered with thin aluminum foils of different thicknesses. The resulting absorption curve showed that most of the uncovered activity was due to the β-particles from Ac^{227} (stopped by 4 mg/cm^2 aluminum corresponding to a maximum energy of 46 kev) and the remainder was almost entirely due to α-particles. All these facts are consistent with the sources being initially radiochemically pure Ac^{227}.

(*b*) *Irradiations*

Fifty-microliter aliquots (approximately 3×10^{-9} g Ac^{227}) of the stock solutions were pipetted into small silica ampoules. Some of these were sealed directly, but those to be irradiated under cadmium and one other were taken to dryness and were then sealed except for a capillary vent. Cobalt monitors were attached and the samples were irradiated for between 5400 ± 2 seconds and 5417 ± 2 seconds as described by Cabell (3).

(*c*) *Chemical Purification of Irradiated Actinium*

Cation and anion exchange chromatography were used to purify the irradiated actinium.

Each cation column consisted of 1.00 g (dry weight) of 200–400 mesh Dowex 50 (hydrogen form) contained in a glass tube 10 cm long and 6 mm in diameter sealed onto a reservoir 10 cm long and 3 cm in diameter. Both column and reservoir were jacketed at 60°. Before they were loaded, the columns were prepared by washing with water.

Each anion column contained 0.25 g of 200–400 mesh Dowex 1 (chloride form) in a tube 6 cm long and 6 mm in diameter sealed onto a reservoir 5 cm long and 1.5 cm in diameter. The anion columns, which were operated at room temperature, were washed with 5 ml of 10 *M* hydrochloric acid before loading.

The irradiated actinium sample was dissolved in 5 ml of 2 *M* hydrochloric acid and the solution was warmed to 80° before it was fed to a cation exchange column without application of pressure. When the feed had passed, the column was eluted successively with 5 ml of hot 2 *M* hydrochloric acid (to remove bismuth, lead, and francium) and 20 ml of hot 3 *M* nitric acid (to remove radium). Finally the column was eluted with 25 ml of hot 6 *M* nitric acid; the first 5 ml of eluate was rejected and the remainder, which contained the purified actinium, was collected in a centrifuge tube. The Th^{227} daughter of the actinium was left on the column.

The progress of this separation is illustrated in Fig. 1 (each peak was identified by separate experiments using only one of the five components each time).

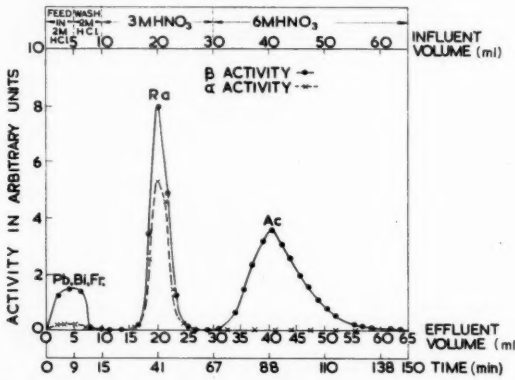


FIG. 1. The separation of Ac^{227} from its daughters by cation exchange chromatography.

Three drops of a ferric nitrate solution (10 mg iron per ml) were added to the eluate obtained from the cation column; the solution was neutralized with ammonia, cooled to room temperature, and the ferric hydroxide precipitate, which carries the actinium, was collected by centrifuging. The precipitate was dissolved in 2 drops of concentrated hydrochloric acid, diluted to 20 ml, and the iron was precipitated again with ammonia. This solution–precipitation cycle was then repeated.

The precipitate was next dissolved in 1 ml of concentrated hydrochloric acid and fed to an anion exchange column at the rate of 0.2 ml/min to remove the iron carrier. After the feed had passed, the column was eluted with 1 ml of 10 *M* hydrochloric acid and the total eluate was collected in a small platinum crucible and evaporated to dryness under an infrared lamp. The crucible was then heated in a furnace at 700° for 5 minutes, cooled, and 2 ml concentrated nitric acid was added and the solution was again evaporated to dryness under an infrared lamp. Finally the residue, which was invisible to the naked eye, was dissolved in 1 ml concentrated nitric acid and the resulting solution was used for making sources on thin films for counting.

(*d*) *Absolute Disintegration-rate Determinations*

(*1*) *Determination of* Ac^{227}

Ac^{227} decays largely by the emission of β-particles of maximum energy 46 kev (α-particle emission is $1.25 \pm 0.02\%$, Peterson and Ghiorso (5)) and initially attempts were made to determine this isotope by absolute β-disintegration-rate determinations. A series of gold-coated VYNS films of known thickness were prepared and a source of freshly purified

Ac^{227} was mounted on a film 36 $\mu g/cm^2$ thick. This was β-counted in a 4π counter and then another film was made to adhere to the back of the source before counting again. Additional films were then added, with counting after each addition, until a final total thickness of 260 $\mu g/cm^2$ had been built up. Over this range, 36 to 260 $\mu g/cm^2$, there was a loss of 12% in the measured β-disintegration rate. Since this method of determination is so sensitive to the thickness of the source mount and extrapolation from 36 $\mu g/cm^2$ to zero thickness is uncertain, it was not investigated further.

The measurement of the α-disintegration rates of sources of the α-active daughters of Ac^{227} is not subject to the same difficulties as a second and similar experiment showed. A source of initially pure Ac^{227} which had been prepared 22 days previously was investigated by the same procedure. It was found that over a source mount thickness varied from 37 to 252 $\mu g/cm^2$ there was no change in α-disintegration rate outside the statistical error of each count, i.e. $\pm 0.3\%$.

Since the half-lives of Th^{227} and Ra^{223} are very much greater than those of the other α-particle-emitting daughters of Ac^{227}, for some purposes the sequence of decay by α-particle emission only may be simplified to:

$$Ac^{227} \xrightarrow[\lambda_1]{\beta} Th^{227} \xrightarrow[\lambda_2]{\alpha} Ra^{223} \xrightarrow[\lambda_3]{4\alpha} Pb^{207}\ \text{(stable)}$$

where λ_1, λ_2, and λ_3 are the decay constants of Ac^{227}, Th^{227}, and Ra^{223} respectively. It can be shown that, with this simplification, the α-activity of an initially pure source containing N_0 atoms of Ac^{227} at time t hours after its preparation is given by the expression:

$$[1] \qquad \frac{-d\alpha}{dt} = \frac{N_0\lambda_1\lambda_2}{(\lambda_2-\lambda_1)}\left(e^{-\lambda_1 t}-e^{-\lambda_2 t}+\frac{4\lambda_3(e^{-\lambda_1 t}-e^{-\lambda_3 t})}{(\lambda_3-\lambda_1)}+\frac{4\lambda_3(e^{-\lambda_3 t}-e^{-\lambda_2 t})}{(\lambda_3-\lambda_2)}\right).$$

Since

$$\lambda_1 = (3.66\pm 0.07)\times 10^{-6}\ \text{hours}^{-1}\ \text{(Tobailem (6))},$$
$$\lambda_2 = (1.590\pm 0.007)\times 10^{-3}\ \text{hours}^{-1},$$

and

$$\lambda_3 = (2.472\pm 0.011)\times 10^{-3}\ \text{hours}^{-1}\ \text{(Hagee, Curtis, and Grove (7))};$$

substitution in expression [1] gives:

$$[2] \quad (-d\alpha/dt)\,.\,1/N_0 = 3.670\times 10^{-6}[e^{-\lambda_1 t}-e^{-\lambda_2 t}+4.006(e^{-\lambda_1 t}-e^{-\lambda_3 t})+11.21(e^{-\lambda_3 t}-e^{-\lambda_2 t})]$$

where $-d\alpha/dt$ is expressed in disintegrations per hour.

Expression [2] may be used to calculate N_0 provided there is no loss of Em^{219} ($t_{\frac{1}{2}} = 3.92$ seconds) from the source. The possibility of this was studied in separate experiments using "weightless" sources in a 4π proportional counter. One source was sealed in the counter for several days after which it was α-counted, then removed, and the α-background of the counter was followed for the next 8 hours. The background decayed with a half-life of 35 minutes, showing the presence of Bi^{211} (daughter of Pb^{211}). In a second experiment a high gas flow was passed through the counter into a second and larger counter whose rise in α-counting rate was measured. From these and similar experiments it was concluded that up to 2% of the Em^{219} content of a thin source could diffuse into the counting gas. It was found that any error introduced into the determination of total α-disintegration rate by this effect could be reduced to negligible proportions ($< 0.5\%$) by sealing the source in the counter for at least three hours before measurement and by using a minimum gas flow during the measurement.

The possibility of loss of Ra^{223} (and consequently of subsequent decay products) by recoil from the source on emission of an α-particle by Th^{227} must also be considered. Since the recoil energy is 108 kev, the range of Ra^{223} in gold will be of the order 5 $\mu g/cm^2$ and recoils in the direction of the source mount will be successfully trapped, but for a source only 1 atom thick losses of 50% may be expected due to recoil in the opposite direction. In fact it is not possible to make truly "weightless" sources by the method employed in this work, since even the purest reagents inevitably contain traces of non-volatile impurities. The true thickness of the source is seldom known in 4π counting and, in fact, is the largest factor contributing to uncertainty in the technique (see later). In the present work it was known that the recoil effect had no influence on the results. Counting for experiments 3 and 4 of Table II was carried out using sources completely enclosed by gold-coated VYNS films and the results obtained agree with those using uncovered sources. The technique of covering the sources with another thin film was not used except in this one case, since it is clearly unnecessary and additional absorbers should be avoided wherever possible.

Observing these precautions it was found that α-activity measurements made for four or five consecutive days commencing 6 days after preparation of a source gave values for N_0 which agreed with a coefficient of variation of 0.4% to 1.0%. Table I shows the results obtained with one such source.

TABLE I

Determination of Ac^{227} by measurement of its α-particle-emitting daughters

Hours from initial count	α-Count per hour corrected for initial count	$N_0 \times 10^{-10}$
137.1	105920	9.079
159.5	128390	9.098
182.3	151970	9.095
205.8	175800	9.025
231.9	205110	9.050
		Average 9.069 ± 0.032

It is interesting to compare the results obtained by these two possible methods of determining Ac^{227}, i.e. by 4π counting the β-particles emitted and by measurement of the rise in α-particle activity. The former method has been found to give results 23% to 45% lower than the latter for samples prepared from different purifications. These figures emphasize the very large errors which may be expected due to self-absorption when attempts are made to 4π count very weak β-particles even though apparently "weightless" sources are employed.

(*2*) *Determination of* Ac^{228}

The curved line ABC of Fig. 2 shows a decay curve obtained from a purified sample of neutron-irradiated Ac^{227} measured in a 4π proportional counter with its anode potential adjusted for β-particle counting. The shape of the falling portion of the curve, AB, is largely determined by the decay of the β-active Ac^{228} component of the source. By point B most of the Ac^{228} has decayed and the recorded count is largely due to the β-active Ac^{227} component (it has been shown in the previous section that this count cannot be used for the quantitative determination of Ac^{227}).

In addition to the β-particles from Ac^{227} the counter also records pulses arising from α- and β-particles emitted by Th^{227} and its radioactive daughters, and it is the growth of

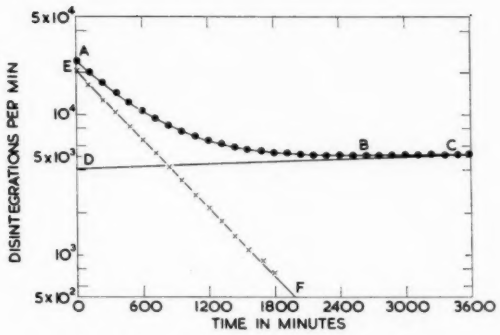


FIG. 2. Decay of neutron-irradiated Ac^{227}.

these which accounts for the upward inclination of the portion of the curve BC. This increase in counting rate follows a form similar to that given in brackets in expression [2], and information obtained by counting after 3 or 4 days (by which time the Ac^{228} will have decayed to about 10^{-5} of its initial amount) was used to correct the portion of the curve AB for these components (line CD). In this way the Ac^{228} contribution alone was obtained and is plotted as points which lie about the line EF which is drawn with a half-life of 6.13 hours, the published value for Ac^{228} (Hahn and Erbacher (8)).

The β-particles emitted by Ac^{228} are mostly highly energetic but 13% have energies below 450 kev (Strominger, Hollander, and Seaborg (9)); sources were therefore examined for possible source mount losses by the technique described above for Ac^{227} particles. It was found that over a source mount thickness varied from 36 to 266 $\mu g/cm^2$ counting rate losses of mixtures of Ac^{227} and Ac^{228} could be ascribed entirely to losses in counting the Ac^{227} component. Using mounts approximately 50 $\mu g/cm^2$ thick losses in counting Ac^{228} would not exceed 0.2% due to this source of error.

A more serious source of error in the absolute disintegration-rate determination of Ac^{228} lies in the possibility of self-absorption in the source itself. Cobble (10) has reported that in certain cases up to 10% losses may occur from apparently "weightless" sources due to this cause and we have shown that for Ac^{227} the losses are even greater. With 450-kev β-particles errors of this magnitude are most unlikely. Since the abundance of the β-particles under consideration is only 13% of the total, we have concluded that the self-absorption losses in determining Ac^{228} in "weightless" sources is much less than one per cent.

(3) Counting Procedure

An important feature of the counting procedure used lay in the fact that after irradiation and purification of the actinium, a single aliquot could be used to determine both the amount of Ac^{227} in that aliquot and also the amount of Ac^{228} which it had yielded on irradiation. Consequently, provided sufficient actinium was purified that adequate counting rates could be obtained, the chemical yield of the purification process was unimportant.

When a batch of irradiated Ac^{227} had been purified, three or four aliquots (sufficient to give between 100 and 1000 β-particle counts/second but whose volume did not need to be known) were mounted on films of approximately 50 $\mu g/cm^2$ thickness and as soon as possible each was counted for α-particles and then, at a higher applied potential, for β-particles in a 4π counter. The progress of β-decay was then followed for 6 or 7 days and

the absolute β-disintegration rate of the Ac^{228} component during each initial count was determined as described above. The decay of this component was also determined to confirm its identity and purity. From the corrected initial count the absolute β-disintegration rate which the Ac^{228} in each source would have had at the end of the irradiation was calculated using 0.1131 ± 0.0005 hours^{-1} (Hahn and Erbacher (8)) for its decay constant.

The α-particle activities of the same sources were measured again 6 days after their preparation and also on each of the 5 succeeding days after this. Increases in α-particle activity over the initial counts made when the sources were first prepared were used to calculate values of N_0 from the expression:

$$[3] \quad [(d\alpha_0/dt)-(d\alpha/dt)]\cdot 1/N_0 = 3.670\times 10^{-6}[1.013(e^{-\lambda_1 t}-e^{-\lambda_2 t})+4.006(e^{-\lambda_1 t}-e^{-\lambda_3 t}) + 11.21(e^{-\lambda_3 t}-e^{-\lambda_2 t})]$$

where $-d\alpha_0/dt$ is the initial counting rate.

Expression [3] differs from expression [2] by a small factor on the right-hand side which arises as follows. The initial α-counts are due to the α-particles emitted in the decay of Ac^{227} to Fr^{223} (plus small activities due to α-active daughters produced during the short time between actinium purification and source counting) and thence to Ra^{223}. Subsequent corrected α-particle counts do not take into account the fact that although all Ac^{227} atoms which disintegrate decay eventually to Ra^{223}, only $98.75 \pm 0.02\%$ (Peterson and Ghiorso (5)) of them decay via Th^{227}. The factor $(e^{-\lambda_1 t}-e^{-\lambda_2 t})$, which expresses the Th^{227} contribution to the total α-particle activity, must therefore be corrected accordingly.

Use of expression [3] rather than [2] for calculations has the merit that a short time may be allowed to elapse between actinium purification and counting without prejudicing the result. This is an advantage when several sources from the same purification have to be counted and, moreover, when it is not possible to determine exactly when the actinium was free from daughters.

For each source the values for N_0 determined on 5 successive days by using expression [3] were averaged and the ratio $(R_{\beta,\,N_0})$ was determined for each film where

$$[4] \quad R_{\beta,\,N_0} = \frac{\text{absolute disintegration rate of the } Ac^{228} \text{ in the source at the end of the irradiation}}{\text{number of atoms of } Ac^{227} \text{ in the source}}.$$

Standard deviations for the three or four separate determinations of $R_{\beta,\,N_0}$ for any one irradiation were calculated and additional uncertainties of 1% and 1.9% were allowed for possible errors in β-disintegration-rate determinations and for errors in the half-lives used in calculating N_0. Distinguishing the results for unshielded and shielded samples by the subscripts 1 and 2 respectively, errors in $(R_{\beta,\,N_0})_1$ varied between 2.6% and 4.2% and errors in $(R_{\beta,\,N_0})_2$ between 5% and 16%.

(4) Measurements with Cobalt Monitors

These have been described by Cabell (3) and by Cabell, Eastwood, and Campion (4).

RESULTS

The results were calculated in terms of the convention advocated by Westcott, Walker, and Alexander (11); application of this convention to the type of experiment described in this paper has been described by Eastwood *et al.* (12).

The measured rate of reaction of a detector, R, is expressed as

$$[5] \quad R = \hat{\sigma}.(nv_0)$$

where (nv_0) is the conventional flux, i.e. n is the total neutron density in neutrons/cm^3 and v_0 is 2200 m/sec, and $\hat{\sigma}$, the effective cross section, is defined by expression [5]. It is assumed that the neutron spectrum is the sum of two components; an epithermal dE/E flux distribution cut off at a suitable lower limit of energy and a Maxwellian distribution of thermal neutrons at a temperature $T°$ K. The two components overlap in energy since no upper limit is imposed on the Maxwellian component.

It is possible to express $\hat{\sigma}$ as

$$\hat{\sigma} = \sigma_0(g+rs) \qquad [6]$$

where r is a measure of the relative density of the epithermal component of the neutron spectrum and g and s are functions of the temperature T which describe the departure of the detector from the $1/v$ law; for true $1/v$ behavior $g = 1$ and $s = 0$.

Values of s and g for a number of elements and isotopes for which differential and resonance integral data are well known have been tabulated by Westcott (13) for a number of temperatures. Amongst these is cobalt.

Since the neutron temperatures are not known in the experiments described here, s is replaced by a temperature-independent term, s_0, such that $s_0 = s\sqrt{(T_0/T)}$. Substitution in equation [6] then gives

$$\hat{\sigma} = \sigma_0\{g+r\sqrt{(T/T_0)}s_0\}. \qquad [7]$$

For the cobalt monitors $\sigma_0 = 36.5$ barns, $g = 1$, $r\sqrt{(T/T_0)}$ can be obtained from $(R_{Cd})_{Co}$, the cadmium ratio for Co^{59}, and $s_0 = 0.953$ (Westcott (13)). Therefore, $\hat{\sigma}$ for cobalt can be calculated and substituted in equation [5] to obtain the total flux (nv_0).

Given (nv_0) equation [5] is used to obtain $\hat{\sigma}$ for Ac^{227} and, *if the assumption is made that $g = 1$ for actinium, i.e. that actinium is a $1/v$ absorber in the thermal region*, σ_0 for actinium may then be obtained from equation [7].

The resonance integral for actinium, $(\text{R.I.})_{Ac}$, is given by:

$$(\text{R.I.})_{Ac} = (\text{R.I.})_{Co}\frac{(R_{Cd}-1)_{Co}}{(R_{Cd}-1)_{Ac}}\cdot\frac{(\sigma_0)_{Ac}}{(\sigma_0)_{Co}}. \qquad [8]$$

We have used 48.6 barns for the resonance integral of Co^{59}.

The results obtained are recorded in Table II. It is not to be expected that $\hat{\sigma}(Ac^{227})$ will be constant since the experiments extended over a period of months during which the neutron spectrum in the irradiation position (and hence R_{Cd}) changed. An average value of 839 barns was obtained for $\hat{\sigma}(Ac^{227})$.

The four values of $\sigma_0(Ac^{227})$ recorded in Table II were each weighted in proportion to the reciprocal of the square of its error and gave an average value of 814 ± 13 barns. Similar treatment of the four results for $(\text{R.I.})_{Ac}$ gave an average value of 1177 ± 19 barns.

CONCLUSIONS

The thermal capture cross section of Ac^{227} obtained in this work (814 ± 13 barns) is considerably larger than any figure previously obtained. It is not possible to explain why this figure differs from those quoted by Hughes and Schwartz (1) (510 ± 40 barns by a pile oscillator method and 520 ± 50 barns by an activation method) since these authors refer to unpublished work. Timma and Russell (2) give details of their work which led to 493 ± 53 barns, using an oscillator method, and, although no obvious flaw is apparent in their procedure, the possibility of neutron attenuation within the sample does not

TABLE II

Results of integral cross section determinations

	Experiments 1 and 2		Experiments 3 and 4		Experiments 5 and 6		Experiments 5 and 7	
	With cadmium cover	Without cadmium cover	With cadmium cover	Without cadmium cover	With cadmium cover	Without cadmium cover	With cadmium cover	Without cadmium cover
$(R_{\beta,N_0})/(1-e^{-\lambda t})$ = Equilibrium rate of production of Ac^{228} from Ac^{227} (sec^{-1})	$(2.12\pm0.17)\times10^{-9}$	$(5.44\pm0.14)\times10^{-8}$	$(2.22\pm0.11)\times10^{-9}$	$(6.26\pm0.27)\times10^{-8}$	$(2.14\pm0.35)\times10^{-9}$	$(6.39\pm0.19)\times10^{-8}$	$(2.14\pm0.35)\times10^{-8}$	$(6.20\pm0.17)\times10^{-8}$
Duration of irradiation (sec) = t	5400 ± 2	5400 ± 2	5417 ± 2	5400 ± 2	5400 ± 2	5400 ± 2	5400 ± 2	5400 ± 2
Co^{60} Activity of monitor (disintegration per sec per mg Co^{59} per sec of irradiation)	3.70 ± 0.04	107.0 ± 0.4	3.84 ± 0.04	118.0 ± 0.5	4.02 ± 0.04	117.0 ± 0.5	4.02 ± 0.04	119.1 ± 0.5
Cd Ratio for Co^{59}	28.93 ± 0.31		30.75 ± 0.33		29.08 ± 0.31		29.60 ± 0.32	
$r\sqrt{(T/T_0)}$	0.0248		0.0233		0.0246		0.0242	
$\hat{\sigma}(Co^{59})$ In barns	37.36 ± 0.01		37.31 ± 0.01		37.36 ± 0.01		37.34 ± 0.01	
nv_0(Neutrons/cm^2 sec)	$(6.71\pm0.08)\times10^{13}$		$(7.41\pm0.09)\times10^{13}$		$(7.34\pm0.08)\times10^{13}$		$(7.47\pm0.09)\times10^{13}$	
$\hat{\sigma}(Ac^{227})$ In barns	811 ± 23		844 ± 37		870 ± 28		829 ± 24	
Cd Ratio for Ac^{227}	25.7 ± 1.5		28.2 ± 1.9		29.8 ± 4.9		28.9 ± 4.7	
$s_0(Ac^{227})$	1.138		1.084		0.916		0.987	
$\sigma_0(Ac^{227})$ In barns	788 ± 23		824 ± 37		851 ± 28		810 ± 25	
Resonance integral of Ac^{227} (from 0.5 ev to ∞) in barns	1185 ± 74		1198 ± 85		1104 ± 189		1104 ± 183	

appear to have been considered. A major uncertainty in using a macro quantity of a rare and highly radioactive material as the sample under investigation might be expected to lie in the chemical purity of the sample, but although Timma and Russell quote no spectrographic or other analyses for the sample they used, they determined its Ac^{227} content by two independent methods (gravimetrically and calorimetrically) with good agreement.

The procedure described here relies entirely on 4π counting methods for the determination of numbers of atoms. The half-lives involved are known with good precision and agreement between published data and those realized during the experiments was excellent. With the present state of our knowledge of 4π counting the major uncertainty lies in the possibility of self-absorption in the source itself. In the experiments described the sources used were invisible to the naked eye and adequate allowance was made for the possibility of self-absorption, taking into account the fact that only relatively energetic particles were of interest. Should these allowances, in fact, be inadequate it is much more likely that the β-counting rather than the α-counting would be in error and consequently the resulting cross section would be higher rather than lower than that quoted here and would make the discrepancy with previous values even greater.

REFERENCES

1. Hughes, D. J. and Schwartz, R. B. Neutron cross sections. Brookhaven National Laboratory Report BNL 325. 2nd ed. U.S. Govt. Printing Office, Washington, D.C. 1958.
2. Timma, D. L. and Russell, L. N. Absorption cross section of actinium-227. Mound Laboratory Report MLM 744. Office of Technical Services, Dept. of Commerce, Washington, D.C. 1952.
3. Cabell, M. J. Can. J. Phys. **36**, 989 (1958).
4. Cabell, M. J., Eastwood, T. A., and Campion, P. J. J. Nuclear Energy, **7**, 81 (1958).
5. Peterson, S. and Ghiorso, A. The transuranic elements. Part II. McGraw-Hill Book Co., Inc., New York. 1949. p. 135.
6. Tobailem, J. J. phys. radium, **16**, 48 (1955).
7. Hagee, G. R., Curtis, M. L., and Grove, G. R. Phys. Rev, **96**, 817A (1954).
8. Hahn, O. and Erbacher, O. Physik Z. **27**, 531 (1926).
9. Strominger, D., Hollander, J. M., and Seaborg, G. T. Rev. Modern Phys. **30** (No. 2), Part II, 585 (1958).
10. Cobble, J. W. Determination of absolute disintegration rate using $4\pi\beta$-proportional γ-scintillation coincidence counting. Paper given at the American Chemical Society, 133rd meeting. San Francisco, Calif. April 13–18, 1958.
11. Westcott, C. H., Walker, W. H., and Alexander, T. K. Second United Nations International Conference on the Peaceful Uses of Atomic Energy, Geneva. September 1–13, 1958. Paper 202.
12. Eastwood, T. A. Second United Nations International Conference on the Peaceful Uses of Atomic Energy, Geneva. September 1–13, 1958. Paper 203.
13. Westcott, C. H. Effective cross section values for well-moderated thermal reactor spectra. Atomic Energy of Canada Ltd. Report CRRP 787, August, 1958.

PREPARATION AND INFRARED SPECTRA OF DIVINYL SULPHIDE, 2-METHYL-1,3-THIOXOLANE, AND 1,4-THIOXANE[1]

K. K. Georgieff and A. Dupré

ABSTRACT

Dehydration of thiodiglycol at 195°–230° with potassium hydroxide produced a 36% yield of divinyl sulphide, 9–10% of 2-methyl-1,3-thioxolane, 7–8% of 1,4-thioxane, and 3.5% of vinyl 2-hydroxyethyl sulphide. Physical properties and infrared spectra are reported. Divinyl sulphide of high purity for polymer work was obtained by distillation in a Podbielniak column. Precautions that must be observed in the preparation, distillation, and storage are given.

INTRODUCTION

To prepare some divinyl sulphide of high purity for polymer and spectroscopic studies, an examination of the possible methods was made. Dehydrochlorination of mustard gas (2,2′-dichlorodiethyl sulphide) gives reasonable yields (1, 2, 3, 4, 5), but this material is very hazardous to handle, and is not readily available commercially. Dehydrobromination of 2,2′-dibromodiethyl sulphide is less dangerous (6) but this material also is unavailable. Dehydration of thiodiglycol with anhydrous potassium sulphate (7) gives some divinyl sulphide, but also a large proportion of by-products.

$$(CH_2OH.CH_2)_2S \rightarrow (CH_2{=}CH)_2S + 2H_2O$$

Dehydration of thiodiglycol with potassium hydroxide, which has been described more recently by Doumani (8), appeared more promising since no mention of by-products other than 2-hydroxyethyl vinyl sulphide was made. Furthermore, since thiodiglycol is commercially available, investigation of this latter method appeared justified.

Doumani added thiodiglycol slowly to potassium hydroxide and continuously distilled off the divinyl sulphide and water. In this investigation, all of both reagents were added at the beginning and were stirred, but whether this difference in procedure materially affected the yield of divinyl sulphide is not known, since Doumani failed to publish such data. No systematic investigation to determine optimum yield conditions was made, but some precautions which are necessary due to excessive foaming of the thiodiglycol and the ease of polymerization of divinyl sulphide are described in the experimental section.

EXPERIMENTAL

Samples of thiodiglycol were kindly supplied by the Defence Research Laboratories, Shirley Bay, Ontario, and Carbide and Carbon Chemicals Corp., and were used without prior purification. Foaming during the reaction is a problem but may be largely overcome if the following precautions are observed: an oversize reaction flask fitted with a stirrer should be used and the temperature of reaction should be raised very slowly. Also, if it is desired to prepare a second batch, the flask must be thoroughly cleaned before a new charge is added, since even small amounts of residue cause uncontrollable foaming.

The reaction was carried out as follows: into a 500-ml round-bottom flask fitted with an Ace "Tru-bore" stirrer and a distillation column head with a water-cooled condenser were placed 75 ml of thiodiglycol and 75 g of potassium hydroxide pellets (85%). To minimize polymerization, the entire apparatus was evacuated and filled with nitrogen.

[1]*Manuscript received February 17, 1959.*
Contribution from the Research Laboratories, Shawinigan Chemicals Ltd., Shawinigan, Quebec.

The stirrer was started after adjusting the blade so as to just clear the pellets, and the flask was immersed in a bath of Arochlor 1252. The bath temperature was raised slowly and reaction started at 195°. The organic vapors and water were condensed and drawn off as soon as they formed. Reaction was complete at 230°. The product, which was kept at −50° during the course of the reaction, was allowed to warm up to room temperature and the organic layer separated from the water. The aqueous layer was not further worked up even though it contained several per cent of product. The organic layer was dried with Linde Molecular Sieves No. 4A, stabilized with 1% para-tertiary butylcatechol and 2% copper resinate, and fractionated at a pressure of 105–156 mm Hg (abs.) in a Podbielniak column which had previously been flushed with nitrogen. The distillate receivers were kept at −50° during the distillation.

Since divinyl sulphide polymerizes rapidly at room temperature in the presence of air, the contents of each Podbielniak receiver was redistilled as soon as possible into a closed tube fitted with a stopcock and semiball joint using the high vacuum, low temperature technique (9, 10). By storing the samples in the above tubes at −78° in the complete absence of permanent gases, especially oxygen, it was possible to keep over 90% of the material in a monomeric state for over a year without the use of any stabilizer. Traces of certain impurities markedly decrease the rate of polymerization. For example, purified divinyl sulphide recovered from raw acetylene produced from calcium carbide, which probably still contained minute traces of divinylacetylene, did not polymerize at all under the above conditions in over a year.

Preparation of 2-Methyl-1,3-thioxolane from 2-Mercaptoethanol and Acetaldehyde (11)

Redistilled 2-mercaptoethanol (31.2 g) (obtained from Fisher Scientific Co.) and 17.7 g of freshly distilled acetaldehyde dissolved in 100 ml of cold benzene were charged into a 250-ml, stirred, round-bottom flask, fitted with a distillation column head with a brine-cooled condenser. Some heat was generated on mixing the reagents. Then 8 ml of 0.7 *N* anhydrous hydrogen chloride in ether was added from a dropping funnel over a period of 5 minutes. Slight cooling was required to maintain a temperature of 35–38°. The charge was then heated slowly to the reflux point and maintained there for 4 to 5 hours until the theoretical amount of water formed and was drawn off. The crude product was cooled and extracted with several 10-ml portions of aqueous 10% potassium carbonate solution. The benzene was separated by distillation through a short column at atmospheric pressure and the 2-methyl-1,3-thioxolane was fractionated in a Podbielniak column at 53 mm Hg (abs.). Yields were 77–80% on acetaldehyde and somewhat higher on mercaptoethanol.

Infrared spectra were obtained with a Perkin–Elmer, Model 12-C, single beam spectrometer with a rock salt prism.

RESULTS

The dehydration of thiodiglycol with potassium hydroxide produced a much more complex reaction product than previously indicated (8). After the mixture was separated into several main fractions by distillation in a Podbielniak column, the fractions were further resolved by gas chromatography. The various components so obtained were then identified by their mass, infrared, and ultraviolet spectra. The yield of divinyl sulphide was about 36%. Its spectra and rate of polymerization indicated that the mid-distillation cut was of high purity. Its infrared spectra in the gas and liquid phases are given in Fig. 1.

All three major by-products showed parent peaks of 104 in the mass spectrometer.

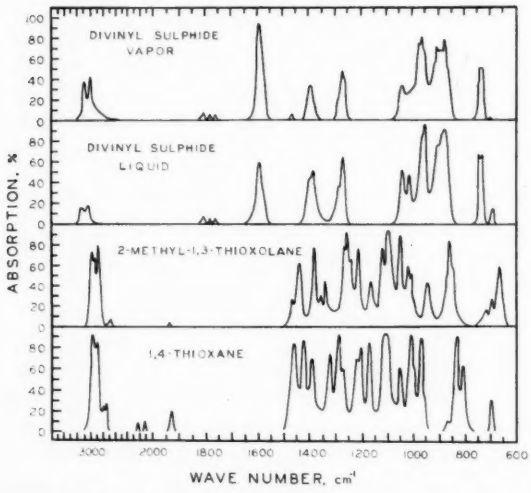


FIG. 1. Infrared spectra. Divinyl sulphide vapor, 34.4 mm pressure in 10-cm cell. Peaks: 3080, 3010, 1806, 1781, 1755, 1592, 1465, 1388, 1286sh, 1272, 1261sh, 1039, 969, 958, 953sh, 914sh, 908, 888, 876, 858sh, 746sh, 738, 733, 696 cm^{-1}.

Divinyl sulphide liquid, 10% by volume in CCl_4 or CS_2 in 0.14-mm cell. Peaks: 3110, 3040, 2970sh, 1588, 1391sh, 1383, 1279, 1265, 1041, 1016, 960sh, 951, 901, 883sh, 874, 736, 729, 685 cm^{-1}.

2-Methyl-1,3-thioxolane, 10% by volume in CCl_4 or CS_2 in 0.128-mm cell. Peaks: 2980, 2940, 2890, 2680, 1934, 1468, 1439, 1380, 1356, 1340, 1267, 1256, 1240, 1212, 1162, 1121, 1095, 1049, 1017, 1007, 996sh, 941, 872sh, 857, 846sh, 712, 691, 661 cm^{-1}.

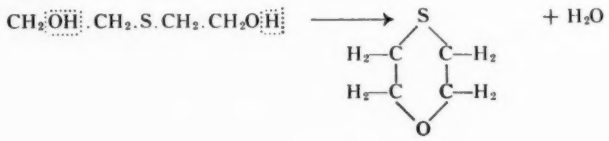
1,4-Thioxane, 100% in 0.029-mm cell. Peaks: 2960, 2900, 2800, 2750, 2220, 2110, 1925, 1459, 1420, 1388, 1323, 1286, 1272, 1216, 1203, 1172, 1105, 1050, 1006, 993sh, 969, 956sh, 865, 828, 809, 693 cm^{-1}.

The highest boiling one appeared to be vinyl 2-hydroxyethyl sulphide in accordance with the findings of Doumani (8), but in point of quantity it was the smallest. The yield was about 3.5%.

$$(CH_2OH.CH_2)_2S \rightarrow CH_2OH.CH_2.S.CH{=}CH_2 + H_2O$$

Infrared bands at 3500 cm^{-1} and at about 1100 indicated the presence of hydroxyl and ones at 1634 and 945 indicated unsaturation.

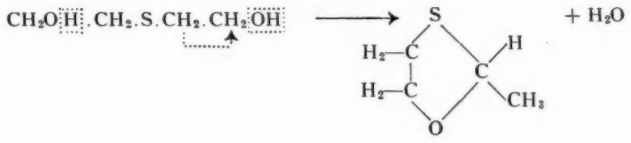
The second highest boiling by-product was identified as 1,4-thioxane (*p*-oxathiane). Physical properties, infrared and mass spectra, were identical with those of an authentic sample (purchased from Robinson Brothers Ltd., Great Britain). It also formed by splitting of 1 mole of water from thiodiglycol but in this case, elements were removed from both hydroxyl groups, followed by cyclization.



Its formation was not surprising since it was previously obtained from thiodiglycol using potassium sulphate (7) or sulphuric acid (12) as dehydrating agents. The yield was 7–8%. Its infrared spectrum is given in Fig. 1.

The major and third highest boiling by-product was unexpected since its formation

from thiodiglycol does not appear to have been previously reported. It too formed by splitting of 1 mole of water. Its mass spectrum showed a parent peak of 104 and large peaks at 15 and 89 which suggested a methyl group. Its infrared spectrum (cf. Fig. 1) failed to give any indication of unsaturation or a hydroxyl group. Since a straight chain compound would require one element of unsaturation, a cyclic compound seemed probable. Since it boiled about 27° below 1,4-thioxane, a five-membered ring instead of a six appeared possible. From the above data and since its boiling point was identical with that reported by Copenhaver (13, 14), it appeared that our compound might be 2-methyl-1,3-thioxolane (2-methyl-1,3-oxathiolane).



This was confirmed by preparing an authentic sample by the reaction of 2-mercaptoethanol with acetaldehyde in the same manner as Kipnis and Ornfelt (11) prepared 2-isopropyl-1,3-thioxolane from 2-mercaptoethanol and isobutyraldehyde. Physical properties, mass and infrared spectra were identical with those of the compound obtained from the dehydration of thiodiglycol. Its yield from thiodiglycol was 9–10%. The compound appeared to be quite stable at 0° for a period of at least one year.

The lowest boiling by-product obtained was methyl vinyl sulphide. It composed 7–8% of the total volatile product. Identification was made by its boiling point, infrared spectrum (15, 16), and mass spectrum. It is probable that it was formed, in part or in whole, by the dehydration of 2-hydroxyethyl methyl sulphide, which appeared to be a likely impurity in the thiodiglycol. Good mass spectra could not be obtained due to their high boiling points, but on distillation of a few drops from a small charge of the original thiodiglycol in a Podbielniak column, very much larger mass peaks of 74, 61, 47, and 15 were obtained with the distillate than with the residue. This is an indication that some 2-hydroxyethyl methyl sulphide was originally present. Two other by-products were obtained in trace quantities. They had mass parent peaks of 100 and 118 but could not be identified.

Some of the physical properties of the compounds obtained are given below.

TABLE I

Compound	B.p.*	n_D^{20}	d_4^{20}
1,4-Thioxane	67° at 44 mm (abs.)	1.5080	1.105
2-Methyl-1,3-thioxolane	58° at 53 mm (abs.) 130.55° (corr.) at 752 mm	1.4867	1.069
Divinyl sulphide	42.5° at 150 mm	1.5076	
Methyl vinyl sulphide	28° at 155 mm		

*All boiling points are uncorrected except when otherwise stated.

ACKNOWLEDGMENTS

The authors wish to express their thanks to Shawinigan Chemicals Ltd. for permission to publish this paper and to Y. Richard, E. Blais, and E. Beaulieu for their assistance.

REFERENCES

1. HELFRICH, O. B. and REID, E. E. J. Am. Chem. Soc. **42**, 1208 (1920).
2. BALES, S. H. and NICKELSON, S. A. J. Chem. Soc. **121**, 2137 (1922).
3. BALES, S. H. and NICKELSON, S. A. J. Chem. Soc. **123**, 2486 (1923).
4. LAWSON, W. E. and REID, E. E. J. Am. Chem. Soc. **47**, 2821 (1925).
5. DAVIES, J. S. H. and OXFORD, A. E. J. Chem. Soc. **133**, 224 (1931).
6. RUIGH, W. L. and ERICKSON, A. E. J. Am. Chem. Soc. **61**, 915 (1939).
7. FROMM, E. and UNGAR, B. Ber. B, **56**, 2286 (1923).
8. DOUMANI, T. F. *To* Union Oil Co. of California. U.S. Pat. No. 2,532,612 (1950).
9. SANDERSON, R. T. Vacuum manipulation of volatile compounds. John Wiley & Sons, Inc., New York. 1948. p. 86.
10. GEORGIEFF, K. K., CAVE, W. T., and BLAIKIE, K. G. J. Am. Chem. Soc. **76**, 5494 (1954).
11. KIPNIS, F. and ORNFELT, J. J. Am. Chem. Soc. **71**, 3555 (1949).
12. HEPWORTH, H. J. Chem. Soc. **119**, 1249 (1921).
13. COPENHAVER, J. W. *To* General Aniline and Film Corporation. Brit. Pat. No. 642,253 (1950).
14. COPENHAVER, J. W. *To* General Aniline and Film Corporation. U.S. Pat. No. 2,551,421 (1951).
15. SCHREIBER, K. C. Anal. Chem. **21**, 1168 (1949).
16. PRICE, C. C. and GILLIS, R. G. J. Am. Chem. Soc. **75**, 4750 (1953).

SELF-ABSORPTION IN SOURCES PREPARED FOR 4π BETA COUNTING[1]

JANET S. MERRITT, J. G. V. TAYLOR, AND P. J. CAMPION

ABSTRACT

Using the $4\pi\beta$-γ coincidence method the self-absorption of $4\pi\beta$ counting sources prepared by a number of source preparation techniques has been measured for the negatron emitters Cs^{134} (86-kev branch only), Nb^{95}, Hg^{203}, Co^{60}, Sc^{46}, Rb^{86}, Au^{198}, Na^{24}, and the positron branch of Cu^{64}. The self-absorption varied from a few tenths of one per cent to about ten per cent for sources with a mean superficial density of 2 $\mu g/cm^2$ for most of the nuclides studied. Subsidiary experiments were carried out to indicate the variation of self-absorption with source superficial density.

INTRODUCTION

The comparative simplicity of $4\pi\beta$ counting has led to its general use for absolute disintegration rate measurements. It is well known that the chief corrections are those required for absorption in the source and mount. The latter has been extensively discussed (1, 2, 3) and is small for all but the softest beta emitters if thin source mounts are used. Self-absorption cannot be so readily determined, and this is the principal uncertainty in $4\pi\beta$ counting. Hawkings *et al.* (4) progressively diluted a stock solution, and observed the activity (expressed in terms of the original solution) as a function of the dilution factor. At sufficiently high dilutions a constant activity was observed, which was taken to indicate that self-absorption had been eliminated. This procedure has been criticized (5) on the grounds that diluting a stock solution may not decrease the size of the crystals forming an active deposit but merely increase the distance between them. Pate and Yaffe (5) have described a distillation technique for preparing uniformly thin sources and have used this method to set up self-absorption curves for several isotopes (6). Seliger and Schwebel (7) have estimated the self-absorption of a number of isotopes from electron microscope measurements of the crystal sizes. The $4\pi\beta$-γ coincidence method has been used by Gunnink *et al.* (8) to study self-absorption in thin sources prepared by conventional techniques. Assuming that the observed inefficiencies are entirely due to self-absorption they have obtained a curve of per cent loss in 4π counting against maximum beta particle energy. These authors are careful to point out that these losses are "best" averages, and that the value for any one source may deviate considerably from the mean.

In this laboratory a $4\pi\beta$-γ coincidence counting system consisting of a $4\pi\beta$ proportional counter sandwiched between two 3 in. $\times$ 3 in. sodium iodide crystals (9) has been used to investigate the relative merits of various source preparation techniques. The results of six such techniques applied to several isotopes of widely differing maximum beta particle energy are reported here.

EXPERIMENTAL

In their simplest form the equations relating the observed counting rates in a coincidence determination to the absolute disintegration rate, N_0, are:

$$N_\beta = N_0\epsilon_\beta$$
$$N_\gamma = N_0\epsilon_\gamma$$
$$N_c = N_0\epsilon_\beta\epsilon_\gamma$$

[1]*Manuscript received March 11, 1959.*
Contribution from the Physics Division, Atomic Energy of Canada Limited, Chalk River, Ontario.
Issued as A.E.C.L. No. 796.

where N_β, N_γ, and N_c are the observed rates in the beta, gamma, and coincidence scalers respectively, and ϵ_β and ϵ_γ are the efficiencies for the beta and gamma detectors. Hence it follows that

$$\epsilon_\beta = N_c/N_\gamma.$$

In practice these equations require several small corrections, the largest of which are due to dead time, accidental coincidences, and backgrounds (9). Since the counter has been shown to have 100% of 4π geometry (9), the inefficiency in the beta detector is due to self-absorption, source mount absorption, and the possibility that the counter–amplifier system may not detect one ion pair.

The following negatron emitters Cs^{134} (86-kev branch only), Nb^{95}, Hg^{203}, Co^{60}, Sc^{46}, Rb^{86}, Au^{198}, Na^{24}, and the positron branch of Cu^{64} were used in this study. Aliquots were deposited on VYNS plastic films (superficial density 5 $\mu g/cm^2$) stretched across aluminum rings 1.5 in. outside diameter, 1.0 in. inside diameter, and 0.022 in. thick. The underside of the plastic film was coated with 15 $\mu g/cm^2$ of gold. After a sample was deposited, a similar gold-coated film was placed over the source so that both gold surfaces were outermost. Provided that the source is not exceptionally thick this second film adheres well to the source material and the first film without forming cavities. The resistance between the center of a film and the mount ring on the coated and uncoated side is about 0.2 and 1.5 meg respectively, while the resistance of a film having no gold on either side is greater than 600 meg. This sandwich technique ensures that no active material can flake off the source, and also provides a more durable source mount for very little increase in total mount thickness compared with a single film coated on both sides.

The correction for absorption in the source mount was determined by observing the efficiency as a function of the number of pairs of gold-coated films added to the sandwich, and extrapolating the resultant "absorption curve" to zero film thickness. The corrections are listed in Table I, those for Cs^{134}, Nb^{95}, Co^{60}, and Na^{24} were measured as described above, while the remainder were found by interpolation.

A small correction required for the threshold detection energy of the counter–amplifier system was estimated as follows. Pulses due to K X rays from Ca^{41}, Cr^{51}, and Fe^{55} observed at the output of the first stage of the amplifier showed that the system was linear in the range 3.3 to 5.9 kev. Comparison of these pulses with the amplitudes of suitably shaped signals fed to the amplifier input from a pulse generator which just failed to register in the beta scaler gave a threshold detection energy of 0.35 ± 0.1 kev when the counter was operated near the center of the plateau. Assuming that self-absorption does not appreciably distort the spectrum, corrections for undetected particles below this threshold energy were calculated using theoretical beta spectral shapes. The corrections for this effect are shown in Table I.

The determination of the efficiency for the 86-kev beta branch in Cs^{134} presented some difficulty owing to the complex nature of the Cs^{134} disintegration scheme. The results for this branch were determined by accepting pulses in the γ-channel corresponding to the sum peak at 1.97 Mev only. In order to increase the efficiency for detecting this peak the outputs of both crystals were added linearly before discrimination, giving a gamma efficiency of 0.03% (as compared with 10 to 15% for the other nuclides). The statistical uncertainty of the efficiency measurement of any one source is estimated to be $\pm 2\%$ for Cs^{134}, while for all the remaining nuclides the corresponding value is $\pm 0.2\%$. The values obtained for the positron branch in Cu^{64} were determined by accepting pulses corresponding to the 0.511-Mev photo peak in the gamma detectors. A correction

of about 0.3% was necessary for the contribution of the 1.34-Mev gamma ray lying under this peak.

To obtain an indication of the variation of self-absorption with source thickness the beta counting efficiency was measured as a function of the carrier concentration for Au^{198}, Rb^{86}, and Nb^{95}. The results are given in Fig. 1 where the crosshatched area represents the spread in self-absorption which was found.

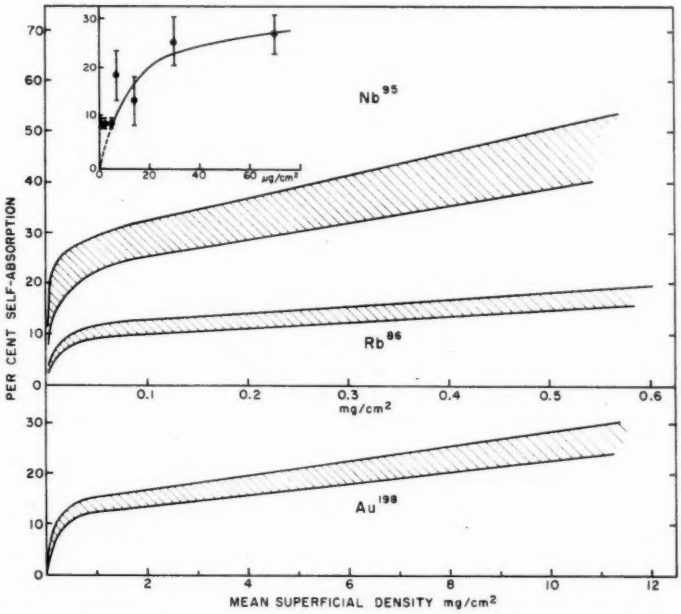


FIG. 1. Per cent self-absorption as a function of the mean superficial density for Au^{198}, Rb^{86}, and Nb^{95}. The Au^{198} and Rb^{86} sources were prepared using the colloidal silica and insulin treatments respectively, while both methods were used in obtaining the curve for Nb^{95}. The inset shows the variation of self-absorption at low mean superficial densities for Nb^{95}.

In order to compare the self-absorption of sources prepared by different methods and with different isotopes, an attempt was made to maintain a mean superficial density of 2 $\mu g/cm^2$, but variations in the area of the deposits may have caused deviations of 50% in this value. In Table I we list the self-absorption, corrected for source mount absorption and threshold detection energy, for a number of isotopes and source preparation techniques. The quoted errors are standard deviations for about five sources. Brief descriptions of, and comments on, the techniques follow according to the column headings of Table I.

(*a*) *Simple evaporation method.*—Aliquots are dried under an infrared lamp and yield a roughly circular deposit of about 1 cm^2. Microscopic inspection shows that this method often results in clumping of the source material, usually around the edge of the deposit, cf. Pate and Yaffe (5). The large errors in this column show the poor reproducibility of the method, especially for the lower energy beta emitters.

(*b*) *Precipitation method.*—A drop of distilled water is added to a source which has been dried as in (*a*). The source is then redried in an atmosphere of ammonia provided by rinsing a beaker with concentrated ammonium hydroxide solution and inverting it

TABLE I

Per cent self-absorption

Nuclide	Maximum beta energy, Mev	Source mount absorption correction, %	Threshold detection energy correction, %	Self-absorption, %					
				Evaporated (*a*)	Precipitated (*b*)	Insulin-treated (*c*)	"Ludox"-treated (*d*)	Sprayed (*e*)	Distilled (*f*)
Cs^{134}	0.086	5	1.1	39±6		21.4±3.2	18.7±1.3	6.7±0.9	
Nb^{95}	0.16	2	0.5	20.3±2.1		9.3±3.5	8.5±1.0		
Hg^{203}	0.21	2	0.5		5.6±0.6		4.0±0.5		
Co^{60}	0.31	1	0.2	8.7±1.5	3.7±0.5	5.2±1.2	3.6±0.7	1.1±0.6	1.3±0.5
Sc^{46}	0.36	0.7	0.2	5.3±1.7	2.8±0.3	5.1±1.4	2.8±0.4		
Cu^{64}	0.66	0.0	0.00	0.2±0.2			0.1±0.2		
Rb^{86}	0.68	0.3	0.08	2.7±1.1			2.3±0.6	1.0±0.2	
Au^{198}	0.96	0.2	0.06	1.6±0.6		1.4±0.6	0.6±0.4		
Na^{24}	1.39	0.04	0.01	1.0±0.5			0.8±0.3	0.2±0.2	

over the source. These sources appear more uniform and exhibit higher counting efficiencies than similar sources prepared by method (*a*). The ammonia treatment is limited to elements which form insoluble hydroxides, but the technique has been extended by the use of hydrogen sulphide in the preparation of precipitated Hg^{203} sources.

(*c*) *Insulin treatment.*—Insulin solution used as a wetting agent improves the uniformity of evaporated sources and hence their efficiencies, particularly when the solids content is high. Although the usual practice is to remove (prior to depositing the activity) most of the insulin solution from the source mount the solids in the remainder are often considerably more than those contained in many high specific activity solutions, and hence the presence of these solids partially offsets the beneficial properties of the insulin. The sources employed in these studies contained about 2 μg of such residual solids from the insulin. Hence the mean superficial density of sources listed in column (*c*) of Table I is about 4 $\mu g/cm^2$.

(*d*) *Colloidal silica treatment.**—Aliquots are delivered into a small amount of silica sol on a source mount and dried as in (*a*). A freshly prepared $1:10^4$ aqueous dilution of "Ludox" SM (du Pont) has been found convenient. A study of beta efficiencies as a function of the amount of "Ludox" showed that about 10 to 20 μl of the diluted sol (about 0.2 to 0.4 μg residual silica) is optimum for most isotopes. Although high ionic concentrations should be avoided to prevent gelation of the silica, "Ludox" has been successfully used with solutions as acidic as 0.5 *N*.

(*e*) *Spray method.*—The source preparation technique of Carswell and Milsted (11) has been used to prepare almost invisible sources on gold-coated VYNS mounts.† The activity is dissolved in acetone, which is then introduced into a fine glass capillary ($\sim$ 0.005 in. at the tip). When about 3 kv d-c. potential is applied between the source mount and the tip of the capillary a fine spray is ejected. Conditions are adjusted so that the droplets forming the spray evaporate before they reach the source mount. Although the method is presently not quantitative, the yields can be made better than 90% and the convenience of the method together with the low self-absorption obtained suggests its use for the technique proposed by Seliger (12) for the standardization of beta emitters having no γ-ray in their decay scheme. A thin source is prepared from high specific activity material, and counted. The deposit is then completely dissolved,

**This method was developed by W. F. Merritt, who used it to prepare* S^{35} *sources (10).*

†*We are indebted to Drs. Fraser and Milton of this laboratory for the use of their equipment, which is a modification of that described by Carswell and Milsted.*

carrier added, and the solution made up to volume to form a standardized stock solution. This can then be used to calibrate other similar solutions by direct comparison.

(*f*) *Distillation method.*—Some sources of Co^{60} have been prepared by the distillation method of Pate and Yaffe (5).* These are very uniform deposits which exhibit about the same self-absorption as sprayed sources. For the sources studied the yields were low, but according to (5) the method may be made very nearly 100% quantitative.

DISCUSSION

The self-absorption values obtained by the simple evaporation method and by the use of colloidal silica (columns (*a*) and (*d*), Table I) are shown graphically in Fig. 2,

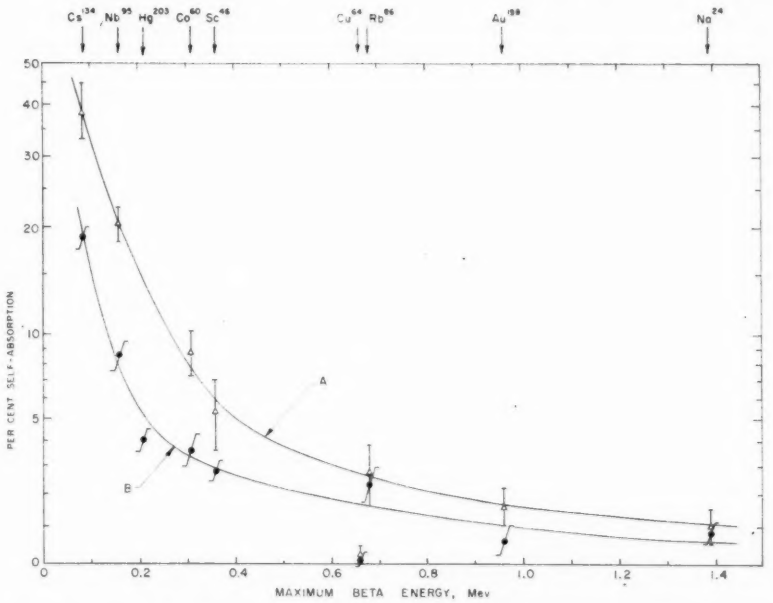


FIG. 2. Per cent self-absorption as a function of beta end point energy for sources of mean superficial density of 2 $\mu g/cm^2$. Curves A and B represent sources prepared by the simple evaporation and colloidal silica methods respectively.

curves A and B respectively. For clarity the results for insulin-treated sources have not been plotted; inspection of Table I shows that they are very similar to those for colloidal silica-treated sources. The results show a comparatively small variation of self-absorption with beta end point energy above about 400 kev, but below this value the absorption rapidly increases. We also note, in agreement with others (8), that the reproducibility in general tends to decrease as the self-absorption increases. On the whole there is good agreement between the self-absorption values obtained by method (*a*) and the experimental results of Gunnink *et al.* (8). Our values are slightly larger than those estimated by Seliger and Schwebel (7), while those obtained for the distilled Co^{60} sources are in agreement with the curve given by Yaffe (6). The use of colloidal silica is seen to be particularly advantageous at low beta end point energies, although no

**We are indebted to Dr. Yaffe and Mr. J. B. Fishman for the preparation of these sources.*

technique that we have investigated to date is capable of reducing self-absorption to negligible proportions. There is one notable deviation from the curves of Fig. 2, viz. the positron branch of Cu^{64}. This may be explained by the fact that owing to the coulomb repulsion effect there are fewer low energy particles in a positron spectrum than in a negatron spectrum having the same end point energy and hence self-absorption effects are smaller.

It should be emphasized that the rapid variation of self-absorption with source thickness (Fig. 1) demands that the source preparation techniques and mean superficial densities be closely similar to those used here if the curves of Fig. 2 are to be used to estimate even approximate self-absorption corrections for $4\pi\beta$ counting. Further, Fig. 1 (inset) and Fig. 8 of (5) show that for sources of smaller mean superficial density it does not necessarily follow that the self-absorption will be significantly less.

REFERENCES

1. SMITH, D. B. A.E.R.E. Rept. I/R 1527 (1954).
2. MANN, W. B. and SELIGER, H. H. J. Research Natl. Bur. Standards, **50**, 197 (1953).
3. PATE, B. D. and YAFFE, L. Can. J. Chem. **33**, 929 (1955).
4. HAWKINGS, R. C., MERRITT, W. F., and CRAVEN, J. H. Proceedings of symposium. Maintenance of Standards, National Physical Laboratory. H.M. Stationery Office, London. 1952.
5. PATE, B. D. and YAFFE, L. Can. J. Chem. **34**, 265 (1956).
6. YAFFE, L. Unpublished results.
7. SELIGER, H. H. and SCHWEBEL, A. Nucleonics, **12** (No. 7), 54 (1954).
8. GUNNINK, R., COLBY, L. J., and COBBLE, J. W. Anal. Chem. To be published.
9. CAMPION, P. J. Intern. J. Appl. Radiation and Isotopes, **4**, 232 (1959).
10. MERRITT, W. F. Unpublished results.
11. CARSWELL, D. J. and MILSTED, J. Nuclear Energy, **4**, 51 (1957).
12. MEASUREMENTS AND STANDARDS OF RADIOACTIVITY. Publication No. 573. National Academy of Sciences, National Research Council, Washington, D.C. p. 47 (1958).

NOTES

INDEPENDENCE IN HYDROLYSIS OF CONDENSED PHOSPHATES

M. JOYCE SMITH

In a series of papers (1) from these laboratories, it was concluded that the hydrolysis of sodium tetrameta-, tetrapoly-, tripoly-, and pyro-phosphate at constant pH and 65.5° C followed first-order kinetics and that hydrolysis of different species in the same dilute solution proceeded independently. A convenient method for making numerical calculations of consecutive reactions was also developed.

When this method was applied to the complete hydrolysis of tetrapolyphosphate near pH 5 using the available rate constants a poor fit was obtained for the curve showing the appearance and disappearance of tripolyphosphate. Either the rate constant for tripolyphosphate at pH 5 or the assumption of independence, or both, might be in error.

Recently an opportunity occurred to investigate the hydrolysis of tetrapolyphosphate using improved chromatographic techniques (2) and carrying out the whole experiment in a reasonably short time without change of personnel.

The results were:

(1) a closer quantitative confirmation of first-order kinetics;
(2) a higher value for the rate constant for tripolyphosphate at pH 5 (6.70×10^{-4} vs. 2.89×10^{-4});
(3) much improved agreement with the theory of consecutive reactions (see Fig. 1); and
(4) confirmation of first-order kinetics by the addition of pyrophosphate and orthophosphate during hydrolysis of tripolyphosphate without effect on the rate constant.

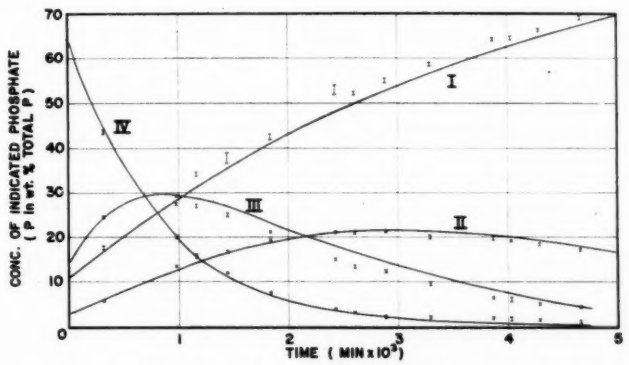


FIG. 1. The course of the reaction degrading tetrapolyphosphate to orthophosphate at 65.5° C and pH = 5.0. Full lines show the proportions of the constituents of the reaction mixture as calculated from the theory of consecutive reactions (1 *d*), individual points show the chromatographically determined values, in duplicate. I, orthophosphate; II, pyrophosphate; III, tripolyphosphate; IV, tetrapolyphosphate.

Since one experiment in the earlier work had given a rate constant of 6.2×10^{-4} at pH 5 for tripolyphosphate, the possibility that it might change drastically in the neigh-

borhood of pH 5 was considered. However, several experiments in the neighborhood of pH 5 showed this not to be the case.

The revised rate constants are given in Table I.

TABLE I

Rate constants for hydrolysis of condensed phosphates (Temp., 65.5° C; Concn., 0.05 at. wt. of P/liter)

Tetrapolyphosphate							
pH				5.0			
$k \times 10^4$				11.7			
Tripolyphosphate							
pH	1.4	3.0		5.0	4.7	4.4	5.3
$k \times 10^4$	86.4	16.2		6.70	7.55	8.40	6.28
Pyrophosphate							
pH	2.8		4.9	5.0			
$k \times 10^4$	4.04		2.99	2.90			

1. (*a*) CROWTHER, J. P. and WESTMAN, A. E. R. Can. J. Chem. **32**, 42 (1954); (*b*) **34**, 969 (1956). (*c*) MCGILVERY, J. D. and CROWTHER, J. P. Can. J. Chem. **32**, 174 (1954). (*d*) WESTMAN, A. E. R. and DELURY, D. B. Can. J. Chem. **34**, 1134 (1956).
2. SMITH, M. J. Anal. Chem. (In press).

RECEIVED FEBRUARY 2, 1959.
ONTARIO RESEARCH FOUNDATION,
43 QUEEN'S PARK,
TORONTO 5, ONTARIO.

THE X-RAY STRUCTURE ANALYSIS OF DEMETHANOL ACONINONE HYDRIODIDE TRIHYDRATE*

MARIA PRZYBYLSKA AND LÉO MARION

The space group of demethanol aconinone hydriodide trihydrate, $C_{24}H_{35}O_8N \cdot HI \cdot 3H_2O$, is $P2_12_12_1$ and unit cell dimensions are $a = 14.69$, $b = 22.08$, and $c = 8.33$ Å, with $Z = 4$.

The co-ordinates of the iodine atom, derived from interatomic vector projections, are $x = 0.167$, $y = 0.209$, and $z = 0.169$ (referred to the origin given by the International Tables).

A total of 2773 structure factors has been evaluated out of a possible 3477 which lie within the range of Cu K_α radiation. The $|F|^2_{hkl}$ values were sharpened and modified as described by Donohue and Trueblood (1) and a three-dimensional Patterson synthesis was computed for 30,000 points. Using the results of this function 'Vector Convergence Density' maps (2) were plotted and a thorough examination of vectors of fourfold coincidence and of Fourier projections gave positions of 32 light atoms, excluding the hydrogen atoms.

The scattering curves only for carbon and iodine atoms were used for calculations of the first (*hk*0) and (0*kl*) structure factors. Two cycles of refinement for these two zones established the locations of the remaining four atoms of the molecule and indicated which atoms should be taken as oxygen atoms.

The ring skeleton of the molecule was found to be identical with that of des-(oxymethyl-

**Issued as N.R.C. No. 5158.*

ene)-lycoctonine (3). The only chemical evidence used in this investigation was that the ethyl group, which was readily identified, is attached to the nitrogen atom (4, 5). This information allowed us to distinguish the nitrogen atom from the other light atoms of the molecule.

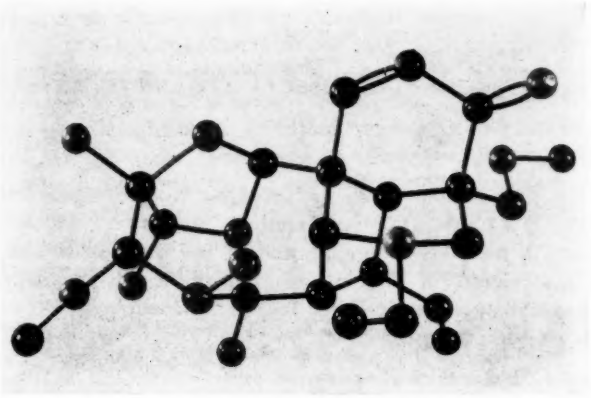
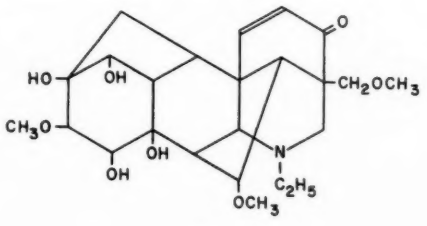


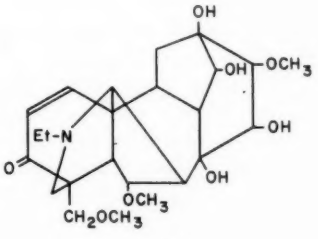
FIG. 1. The molecule of demethanol aconinone.

The calculation of interatomic distances showed the positions of the C=C bond and of the C=O group, which are found to be in agreement with the chemical evidence (6).

The photograph of the molecular model of demethanol aconinone is given in Fig. 1. The structural formula I and the diterpenoid representation of the structure II is also given.



I



II

Refinement of atomic parameters is proceeding. The present values of the reliability index, $R = \Sigma\big||F_o|-|F_c|\big| \div \Sigma|F_o|$, are: $R\,[001] = 0.16$, $R\,[100] = 0.20$, and $R\,[010] = 0.22$.

Full details of this work will be published elsewhere.

We wish to thank Dr. F. R. Ahmed for computational assistance and for evaluating the Patterson function. We are also indebted to Dr. W. H. Barnes for his continued interest in this investigation, to Miss C. Saunderson for her help with the preparation of X-ray data, and to Dr. H. Mayer for supplying authenticated crystals.

1. DONOHUE, J. and TRUEBLOOD, K. N. Acta Cryst. **5**, 414 (1952).
2. BEEVERS, C. A. and ROBERTSON, J. H. Acta Cryst. **3**, 164 (1950).
3. PRZYBYLSKA, M. and MARION, L. Can. J. Chem. **34**, 185 (1956).
4. JACOBS, W. A. and ELDERFIELD, R. C. J. Am. Chem. Soc. **58**, 1059 (1936).

5. MAJIMA, R. and TAMURA, K. Ann. **526**, 116 (1936).
6. MAYER, H. and MARION, L. Can. J. Chem. **37**, 856 (1959).

RECEIVED FEBRUARY 4, 1959.
DIVISION OF PURE CHEMISTRY,
NATIONAL RESEARCH COUNCIL,
OTTAWA, CANADA.

2,4-DIAMINO-2-METHYLPENTANE DIISOCYANATE AND ITS METHYL URETHANE

K. H. KOSSMANN

2,4-Diamino-2-methylpentane is easily prepared from acetone and ammonia via 2,2,4,4-pentamethyl-2,3,4,5-tetrahydropyrimidine ("Acetonine") (1, 2).

While investigating methods of preparation of the diamine it was found desirable to prepare the diisocyanate of diamino-methylpentane and its methylurethane. The procedure used was similar to one given for hexamethylenediamine (3) with the exception that the dihydrochloride was not isolated.

EXPERIMENTAL

A. 2,4-Diamino-2-methylpentane diisocyanate 58 g ($\frac{1}{2}$ mole) of 2,4-diamino-2-methyl-

pentane was dissolved in 500 cc of *sec*-amylbenzene in a 1-liter, 3-necked flask fitted with stirrer, gas inlet, and gas outlet.

Dry HCl gas was bubbled through at 20–25° C, with stirring, until precipitation of the dihydrochloride was complete. The charge was heated with constant stirring to 180–185° C. Phosgene was then introduced, as a gas, for about 16 hours, after which time all the dihydrochloride had disappeared. After filtering, the reaction product was distilled at 10 mm Hg. Diisocyanate (30 g) was obtained, which represented a 35.7% yield. Methylpentanediamine diisocyanate boiled at 90–92° C at 10 mm Hg. Its refractive index at 20° C was 1.4445 (1.4449), its density at 20.5° C was 1.019.

B. The methylurethane of methylpentanediamine diisocyanate was prepared by dissolving 2 g of the diamine in 10 cc of methanol. The mixture warmed up. When it had been cooled the urethane crystallized and was recrystallized from methanol. Its melting point was 81° C.

ACKNOWLEDGMENT

The author wishes to thank Shawinigan Chemicals Limited, Shawinigan Falls, for their permission to publish this note.

1. MATTER, I. E. Helv. Chim. Acta, **31**, 612 (1948).
2. BRADBURY, R. B., HANCOX, N. C., and HATT, H. H. J. Chem. Soc. 1394 (1947).
3. FARLOW, M. W. Org. Syntheses, **31**, 62 (1951).

RECEIVED FEBRUARY 3, 1959.
SHAWINIGAN CHEMICALS LIMITED,
P.O. BOX 330,
SHAWINIGAN FALLS, QUEBEC.

A SIMPLE THERMOSTATED PROBE ASSEMBLY FOR HIGH RESOLUTION NUCLEAR MAGNETIC RESONANCE SPECTRA

S. BROWNSTEIN

The observation of high resolution nuclear magnetic resonance spectra at a variety of temperatures is desirable to determine rates and activation energies for intramolecular motions (2, 3). The problem of constructing a thermostated probe assembly is complicated by the necessity of spinning the sample rapidly, not using magnetic materials, and having only a small space available. Nevertheless two such probes have been described in the literature (1, 4). Both of these assemblies have the disadvantage of requiring highly skilled glass-blowing techniques, and one of these (4) requires winding of a special receiver coil. In the assembly described here only a simple modification of the commercially available receiver coil unit is required. The apparatus may be easily dismantled and therefore can be used at a variety of frequencies by merely exchanging the receiver coil unit.

A plastic sleeve, tapered at the upper end, is cemented to the base of the probe insert. This mechanically strengthens it and serves as a seal to the thermostated compartment. A slot is cut below the receiving coil, and the insert is lengthened by sealing on an additional length of glass tubing. The resultant probe insert is shown in Fig. 1.

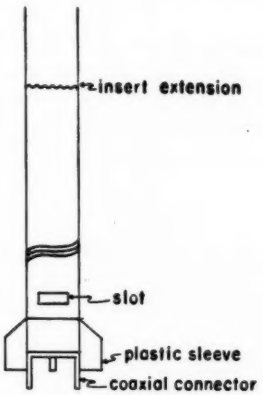
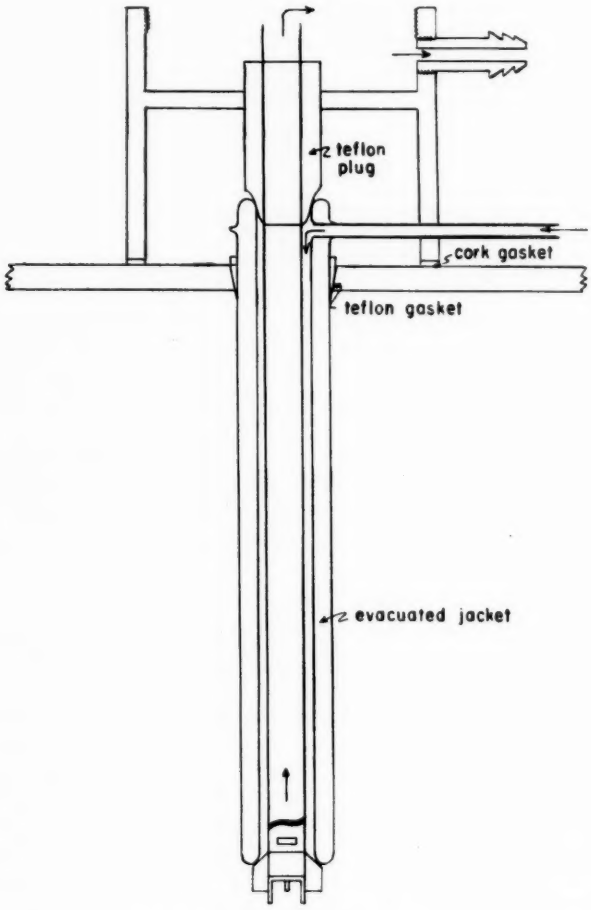


FIG. 1. Modified probe insert.

A cylindrical vacuum jacket, through which a side arm leads, is placed around the insert, fitting against the plastic sleeve at the lower end and supported by a tapered Teflon plug at the upper end. This fits tightly into the probe assembly, with the aid of a Teflon gasket, and is tuned in the usual manner. An aluminum housing, sitting on a cork gasket, with a slot for the glass side arm is screwed to the housing of the probe assembly. An extension of the housing fits snugly against the Teflon plug so that the thermostating air may leave only through the side arm in the aluminum housing. A commercially available sample spinner screws onto the top of the aluminum housing (5). Figure 2 shows the completed assembly with arrows indicating the path of the thermostated air.

The thermostated air was carried to the glass side arm by a Teflon tube wrapped in glass wool and aluminum foil. The temperature was measured by a copper constantan



F.G. 2. Thermostating assembly.

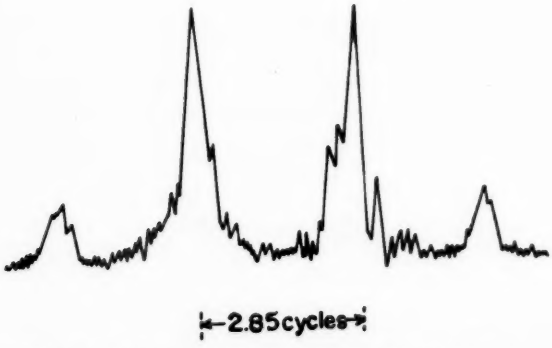


FIG. 3. Resonance of aldehydic proton of acetaldehyde.

thermocouple, B and S gauge 20, inserted through the side arm to 1/4 in. from the receiving coil. This assembly has been operated from −60° to 120° C with resolution equal to that obtained in instruments without a thermostated assembly. Figure 3 shows a trace obtained for the aldehydic proton of acetaldehyde. The resolution is at least one part in 10^8. Since the receiver coil may be tuned without disassembling the apparatus, it is used for routine investigations as well as variable temperature studies.

1. BERNSTEIN, H. J., SCHNEIDER, W. G., and POPLE, J. A. Proc. Roy. Soc. (London), A, **236**, 515 (1956).
2. BOTTINI, A. T. and ROBERTS, J. D. J. Am. Chem. Soc. **78**, 5126 (1956).
3. GUTOWSKY, H. S. and HOLM, C. H. J. Chem. Phys. **25**, 1128 (1956).
4. SHOOLERY, J. N. and ROBERTS, J. D. Rev. Sci. Instr. **28**, 61 (1957).
5. VARIAN ASSOCIATES, Palo Alto, California. Model V-4331A.

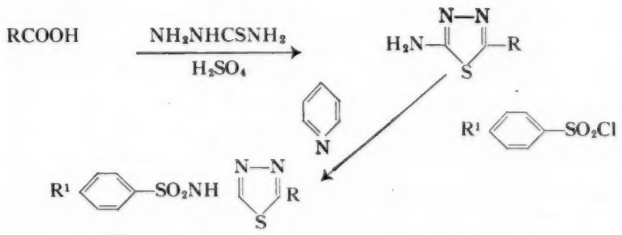
RECEIVED JANUARY 30, 1959.
CHEMISTRY DEPARTMENT,
CORNELL UNIVERSITY,
ITHACA, NEW YORK.

SOME HYPOGLYCEMIC THIADIAZOLES

FRANCIS L. CHUBB AND JACQUELINE NISSENBAUM

In 1942 Janbon (1) first noticed that 2-sulphanilamido-5-isopropyl-1,3,4-thiadiazole caused hypoglycemia. Bovet and Dubost (2) as well as Loubatières (3) studied the relationship between structure and activity in this series. The present work was undertaken in an effort to prepare some new hypoglycemic thiadiazoles.

A series of 2-arylsulphonamido-5-alkyl-1,3,4-thiadiazoles (Table II) was synthesized according to the following reaction scheme.



The pharmacology of these compounds will be reported elsewhere.

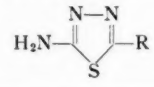
EXPERIMENTAL

2-Amino-5-alkyl-1,3,4-thiadiazoles (*Table I*)

A well-stirred mixture of 0.3 mole of fatty acid, 31.5 ml of concentrated sulphuric acid, and 0.25 mole of thiosemicarbazide was slowly heated to 80–90° and maintained at that temperature for 7 hours. After the reaction mixture was cooled, it was poured into ice water and made basic with concentrated ammonia. The crude product, which precipitated upon addition of the ammonia, was filtered and washed with water. It was recrystallized from alcohol–water.

TABLE I

2-Amino-5-alkyl-1,3,4-thiadiazoles

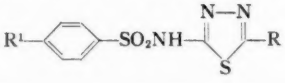


R	M.p., ° C	Yield, %	Formula	Analysis, % Calculated C	Calculated H	Found C	Found H
n-C_3H_7	203–206	80	$C_5H_9N_3S$	*a*			
iso-C_3H_7	182–185	70	$C_5H_9N_3S$	*b*			
n-C_4H_9	194–196	35	$C_6H_{11}N_3S$	*b*			
iso-C_4H_9	228–230	49	$C_6H_{11}N_3S$	*b*			
t-C_4H_9	181–184	54	$C_6H_{11}N_3S$	45.80	7.03	45.84	7.04
sec-C_4H_9	178–180	55	$C_6H_{11}N_3S$	45.80	7.03	45.71	6.82
n-C_5H_{11}	194–196	52	$C_7H_{13}N_3S$	*c*			
iso-C_5H_{11}	214–215	52	$C_7H_{13}N_3S$	49.09	7.59	48.71	7.63

[a]Ohta, M. and Higashijima, T. J. Pharm. Soc. Japan, **72**, 376 (1952).
[b]Wojan, H. and Wuckel, H. Arch. Pharm. **284**, 53 (1951).
[c]Brooks, J. D., Charlton, P. T., Macey, P. E., Peak. D. A., and Short. W. F. J. Chem. Soc. 452 (1950).

TABLE II

2-Arylsulphonamido-5-alkyl-1,3,4-thiadiazoles



R	R^1	M.p., ° C	Yield, %	Formula	Analysis, % Calculated C	Calculated H	Found C	Found H
n-C_3H_7	CH_3	134–136	59	$C_{12}H_{15}O_2N_3S_2$	48.45	5.08	48.62	5.08
n-C_3H_7	OCH_3	125–127	65	$C_{12}H_{15}O_3N_3S_2$	45.98	4.82	46.01	4.81
n-C_3H_7	OC_2H_5	122–124	62	$C_{13}H_{17}O_3N_3S_2$	47.69	5.24	47.69	5.84
iso-C_3H_7	CH_3	119–121[a]	60	$C_{12}H_{15}O_2N_3S_2$	48.45	5.08	48.36	5.12
iso-C_3H_7	OCH_3	141–142[a]	57	$C_{12}H_{15}O_3N_3S_2$	45.98	4.82	45.96	4.64
iso-C_3H_7	OC_2H_5	128–130	65	$C_{13}H_{17}O_3N_3S_2$	47.69	5.24	47.88	5.53
n-C_4H_9	CH_3	115–116	69	$C_{13}H_{17}O_2N_3S_2$	50.13	5.50	49.89	5.75
n-C_4H_9	OCH_3	121–123	74	$C_{13}H_{17}O_3N_3S_2$	47.69	5.24	47.89	4.98
n-C_4H_9	OC_2H_5	112–113	54	$C_{14}H_{19}O_3N_3S_2$	49.25	5.61	49.49	5.74
iso-C_4H_9	CH_3	174–175	50	$C_{13}H_{17}O_2N_3S_2$	50.13	5.50	49.97	5.61
iso-C_4H_9	OCH_3	148–149	68	$C_{13}H_{17}O_3N_3S_2$	47.69	5.24	47.53	4.75
iso-C_4H_9	OC_2H_5	151–152	67	$C_{14}H_{19}O_3N_3S_2$	49.25	5.61	48.81	5.78
iso-C_4H_9	Cl	154–155	44	$C_{12}H_{14}O_2N_3S_2Cl$	43.40	4.25	43.85	4.45
t-C_4H_9	CH_3	148–150	64	$C_{13}H_{17}O_2N_3S_2$	50.13	5.50	49.95	5.68
t-C_4H_9	OCH_3	140–141	74	$C_{13}H_{17}O_3N_3S_2$	47.69	5.24	47.70	5.09
t-C_4H_9	OC_2H_5	161–162	52	$C_{14}H_{19}O_3N_3S_2$	49.25	5.61	49.22	5.52
t-C_4H_9	Cl	163–165	45	$C_{12}H_{14}O_2N_3S_2Cl$	43.40	4.25	43.30	4.48
sec-C_4H_9	CH_3	111–112	40	$C_{13}H_{17}O_2N_3S_2$	50.13	5.50	49.64	5.67
sec-C_4H_9	OCH_3	97–98	40	$C_{13}H_{17}O_3N_3S_2$	47.69	5.24	47.45	4.74
sec-C_4H_9	OC_2H_9	106–107	50	$C_{14}H_{19}O_3N_3S_2$	49.25	5.61	48.80	5.89
n-C_5H_{11}	CH_3	131–132	73	$C_{14}H_{19}O_2N_3S_2$	51.64	5.89	51.64	5.68
n-C_5H_{11}	OCH_3	133–134	65	$C_{14}H_{19}O_3N_3S_2$	49.25	5.61	48.83	5.64
n-C_5H_{11}	OC_2H_5	116–117	57	$C_{15}H_{21}O_3N_3S_2$	50.69	5.96	50.77	5.77
iso-C_5H_{11}	CH_3	140–141	73	$C_{14}H_{19}O_2N_3S_2$	51.64	5.89	51.86	5.72
iso-C_5H_{11}	OCH_3	141–142	65	$C_{14}H_{19}O_3N_3S_2$	49.25	5.61	48.91	5.78
iso-C_5H_{11}	OC_2H_5	99–100	57	$C_{15}H_{21}O_3N_3S_2$	50.69	5.96	50.62	5.89

[a]Ruschig, H., Korger, G., Aumuller, W., Wagner, H., and Weyer, R. Arzneimittel-Forsch. **8**, 448 (1958), have reported melting points for these compounds.

2-Arylsulphonamido-5-alkyl-1,3,4-thiadiazoles (*4*) (*Table II*).

A solution of 0.06 mole of the arylsulphonyl chloride in 25 ml pyridine was slowly added with stirring to a solution of 0.06 mole of 2-amino-5-alkyl-1,3,4-thiadiazole in 25 ml pyridine. The resulting solution, after being allowed to stand overnight at room temperature, was slowly added to an excess of 6 *N* hydrochloric acid. The crude product separated, usually as a solid but sometimes as a yellow gum which crystallized on standing. It was dissolved in dilute sodium hydroxide, treated with charcoal, and reprecipitated with hydrochloric acid. The 2-arylsulphonamido-5-alkyl-1,3,4-thiadiazole was recrystallized from alcohol–water.

Microanalyses were performed by Dr. Carl Tiedcke, Teaneck, N.J., U.S.A.

1. JANBON, M., CHAPTAL, J., VEDEL, A., and SCHAAP, J. Montpellier med. **21–22**, 441 (1942).
2. BOVET, D. and DUBOST, P. Compt. rend soc. biol. **138**, 764 (1944).
3. LOUBATIÈRES, A. Compt. rend. soc. biol. **138**, 830 (1944).
4. DAHLBOM, R. and EKSTRAND, T. Svensk Kem. Tidskr. **55**, 122 (1943).

RECEIVED FEBRUARY 20, 1959.
RESEARCH LABORATORIES,
FRANK W. HORNER LIMITED,
MONTREAL, QUEBEC.

NOTE ON THE PHOTOOXIDATION OF TETRAMETHYLRUBRENE*

ROBIN M. HOCHSTRASSER

Badger's group (1) first noticed that tetramethylrubrene (5,6,11,12-tetra-*p*-tolylnaphthacene) crystals change from their normal deep red to a colorless form on exposure to sunlight. This is not a widely observable phenomenon in as much as substances which are photochemically active when dissolved in a suitable solvent do not normally undergo the same reaction in the pure crystalline form. Rubrene (5,6,11,12-tetraphenylnaphthacene) was found to exhibit the same type of decoloration on exposure to ultraviolet irradiation (3), but in this case specially prepared thin films on a large area substrate were necessary. In each case the product is a transannular peroxide.

It is the purpose of this note to report the results of some experiments on the photooxidation of tetramethylrubrene crystals. The experiments were done at 25° C in cells with plane quartz windows. The light source was a 1-kw AH6 water-cooled mercury arc. The apparatus was as previously described by Hochstrasser and Ritchie (3), and differential pressure measurements could be made to within 0.002 mm Hg at total pressures below 25 mm Hg. The oxidation was followed by noting the changes of oxygen pressure in a constant volume system as the crystals were illuminated with near monochromatic light. The small crystals were scratched on to the surface of a quartz disk (in the absence of absorbable light) and the disk was then carefully sealed on to one end of a cylindrical cell. The cell was then evacuated, oxygen allowed in to a known pressure in the dark, and when stability was maintained the crystals were illuminated and the decrease in pressure determined as a function of time. Experiments involving intermittent illumination and irradiation with light at two wavelengths were performed.

**This research was part of a program supported by a grant from the National Research Council of Canada.*

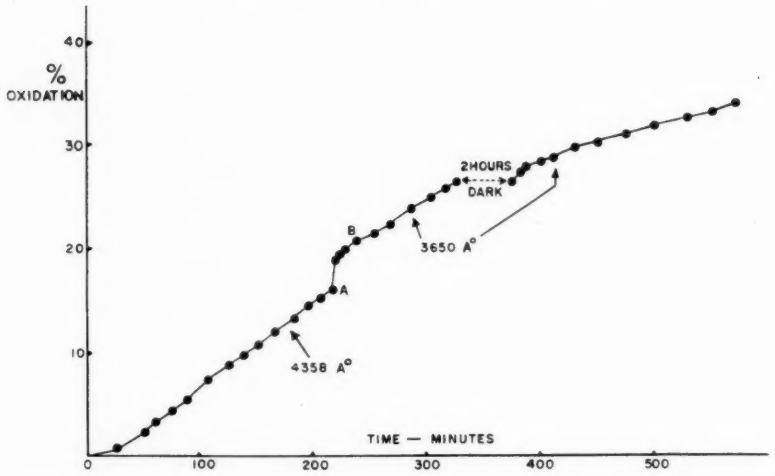


FIG. 1. Photooxidation of tetramethylrubrene crystals (10^{-6} mole of crystal; quanta at 3650 Å approximately double 4358 Å; O_2 pressure 25 mm).

A typical result is shown on Fig. 1 where the extent of the oxidation is plotted against time of irradiation. At A the illumination was discontinued and the crystals left in contact with oxygen for 30 minutes, then reirradiated. The magnitude of the increased rate from A to B was a function of the time of dark contact with oxygen. These results indicate that the photooxidation of tetramethylrubrene is diffusion-controlled. The effects observed when the intensity or the wavelength of the exciting light was changed were again indicative of a diffusional rate-determining step. The absolute rates of oxidation were not reproducible with different crystalline deposits, but the initial rate constants were all of the order of 10^{-10} mole second^{-1} and the quantum yields were approximately 10^{-4} depending on the oxygen pressure. The peroxide is in thermal equilibrium with oxygen but the dissociation is occurring at a negligible rate at this temperature.

Such a system is interesting in as much as not many similar cases are known and certainly not in relation to the combination of molecular oxygen with an aromatic hydrocarbon. As has already been pointed out, crystals of rubrene do not oxidize at a measurable rate even in an atmosphere of oxygen. During the formation of the transannular peroxide a drastic structural change must occur as shown in Fig. 2. Microscopic examination of partly oxidized crystals shows that the oxidation is occurring at the crystal surface.

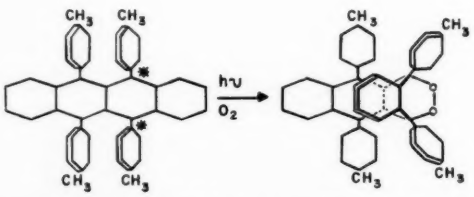


FIG. 2. Schematic representation of the photooxidation of tetramethylrubrene.

It is reasonably well established that oxidations of this type occur via the triplet state of the molecule attained after intersystem-crossing from the first excited singlet state (4). In a molecular crystal intersystem-crossing will manifest itself as localization of excitation energy within the crystal. This arises from the theory of molecular crystals (2) which shows that the singlet levels of the isolated molecule will be split in the crystal into a broad band ($\sim 3000\ cm^{-1}$), while the triplet splitting will be very small by virtue of the fact that the transition moment integral between the ground singlet and first triplet is negligibly small. In order that the reaction with oxygen can occur, this energy must be transported to the surface of the crystal, where there is an oxygen molecule available. It is the availability of oxygen which is the limiting factor in this case. To accomplish photooxidation, the oxygen must first traverse the peroxide layer. Here the rate of diffusion is measurable, and the observation of a diffusion-controlling step suggests that one reason why other aromatic hydrocarbons containing the basic anthracenic structure do not apparently react with oxygen to any great extent is the imperviousness of the peroxide layer to oxygen. With a bulky molecule such as tetramethylrubrene or even rubrene under special conditions this layer becomes permeable. The discovery of an effect such as this opens up a wide field which should yield much information regarding the transport of electronic excitation energy in molecular crystals. In the near future it is proposed to commence a detailed rotating sector and intensity study of the photooxidation of tetramethylrubrene single crystals.

The author expresses his thanks to Professor G. M. Badger for a sample of tetramethylrubrene.

1. Badger, G. M., Pierce, R. S., Rhodda, H. J., and Walker, I. S. J. Chem. Soc. 3151 (1954).
2. Davydov, A. S. J. Exptl. Theoret. Phys. (U.S.S.R.), **18**, 210 (1948).
3. Hochstrasser, R. M. and Ritchie, M. Trans. Faraday Soc. **52**, 1363 (1956).
4. Reid, C. Quart. Rev. **12**, 224 (1958).

Received March 16, 1959.
Department of Chemistry,
The University of British Columbia,
Vancouver, B.C.

CORRECTIONS

Volume 35, 1957

Page 1438. In the title and throughout the paper "Δ^9-1,5,9-triazabicyclo(4.4.0)decene" should read: "Δ^9-hexahydropyrimido(1,2-*a*)pyrimidine" and "Δ^9-1,4,9-triazabicyclo(5.3.0)decene" should read: "Δ^9-hexahydroimidazo(1,2-*a*)-1,3-diazepine".

Volume 36, 1958

Page 1561. In line 3, the equation "$(0.91)^3 = \sqrt{2}.m_2.\sin\alpha$" should read: "$0.91 = \sqrt{2}.m_2.\sin\alpha$".

Pages 1570–1578. The authors of the paper *Theoretical aspects of the unimolecular decomposition of nitrous oxide* have submitted the following:

"In this paper there are certain numerical errors which, although not invalidating the main conclusions, might prove confusing to anyone checking the calculations in detail. These errors stem from an incorrect value of λ_1 (p. 1578), which should be equal to 589.4×10^{26}.

The displacement ratios (pp. 1574 and 1578) should be:

$$\text{for } \nu_1 \quad x:w:y = 1.00:0.685:0$$
$$\text{for } \nu_3 \quad x:w:y = -0.462:1.00:0$$

(those for ν_2 are given correctly). Table I (p. 1574) should be as given below. The product of the μ's (eq. [6], p. 1574) should be 0.4693, and the calculated k_1, with $n = 2$, should be 1.72 cc mole^{-1} sec^{-1}.

TABLE I

i	ν_i, sec^{-1}	α_i	μ_i
1	3.864×10^{13}	1.236×10^{-3}	0.8201
3	6.709×10^{13}	0.8625×10^{-3}	0.5723

On p. 1575 the value of 7.47×10^{-4} sec^{-1} is actually the experimental value of k_∞, not of k^+, which is 8.13×10^{11} sec^{-1}. The value of k_{-1} should have been given as 3.182×10^{-10} cc molecule^{-1} sec^{-1}.

The calculated value of k_1, 1.72 cc mole^{-1} sec^{-1} is still much lower than the experimental value of 14.0 cc mole^{-1} sec^{-1}, so that no change is required in our conclusion that Slater's theory needs modification to permit energy flow between the normal modes of vibration."

Volume 37, 1959

Pages 120–128. Throughout this paper "equivalence" should read "equi-valence" wherever it occurs.

Pages 148–154. Throughout this paper "equivalence" should read "equi-valence" wherever it occurs.

HELVETICA CHIMICA ACTA

SCHWEIZERISCHE
CHEMISCHE GESELLSCHAFT
Verlag Helvetica Chimica Acta
Basel 7 (Schweiz)

Seit 1918 **40** Jahre

Abonnemente: Jahrgang 1959, Vol. XLII $25.00 incl. Porto

Es sind noch lieferbar:

Neudruck ab Lager
Vol. I–XXIV (1918–1941)
Vol. XXV–XXVII (1942–1944) in Vorbereitung.

Originalausgaben, druckfrisch und antiquarisch.
Vol. XXVIII–XLI (1945–1958)

Diverse Einzelhefte ab Vol. XXII
Preise auf Anfrage. Nur solange Vorrat

Das wissenschaftliche Organ der SCHWEIZERISCHEN CHEMISCHEN GESELLSCHAFT

NOTES TO CONTRIBUTORS

Canadian Journal of Chemistry

MANUSCRIPTS

General.—Manuscripts, in English or French, should be typewritten, double spaced, on paper 8½×11 in. **The original and one copy are to be submitted.** Tables and captions for the figures should be placed at the end of the manuscript. Every sheet of the manuscript should be numbered. Style, arrangement, spelling, and abbreviations should conform to the usage of recent numbers of this journal. Greek letters or unusual signs should be written plainly or explained by marginal notes. Characters to be set in bold face type should be indicated by a wavy line below the characters. Superscripts and subscripts must be legible and carefully placed. Manuscripts and illustrations should be carefully checked before they are submitted. Authors will be charged for unnecessary deviations from the usual format and for changes made in the proof that are considered excessive or unnecessary.

Abstract.—An abstract of not more than about 200 words, indicating the scope of the work and the principal findings, is required, except in Notes.

References.—These should be designated in the text by a key number and listed at the end of the paper, with the number, in the order in which they are cited. The form of the citations should be that used in this journal; in references to papers in periodicals, titles should not be given and only initial page numbers are required. The names of periodicals should be abbreviated in the form given in the most recent *List of Periodicals Abstracted by Chemical Abstracts*. All citations should be checked with the original articles and each one referred to in the text by the key number.

Tables.—Tables should be numbered in roman numerals and each table referred to in the text. Titles should always be given but should be brief; column headings should be brief and descriptive matter in the tables confined to a minimum. Vertical rules should not be used. Numerous small tables should be avoided.

ILLUSTRATIONS

General.—All figures (including each figure of the plates) should be numbered consecutively from 1 up, in arabic figures, and each figure referred to in the text. The author's name, title of the paper, and figure number should be written in the lower left corner of the sheets on which the illustrations appear. Captions should not be written on the illustrations.

Line drawings.—Drawings should be carefully made with India ink on white drawing paper, blue tracing paper, or co-ordinate paper ruled in blue only; any co-ordinate lines that are to appear in the reproduction should be ruled in black ink. Paper ruled in green, yellow, or red should not be used. All lines must be of sufficient thickness to reproduce well. Decimal points, periods, and stippled dots must be solid black circles large enough to be reduced if necessary. Letters and numerals should be neatly made, preferably with a stencil (**do NOT use typewriting**), and be of such size that the smallest lettering will not be less than 1 mm high when the figure is reduced to a suitable size. Many drawings are made too large; originals should not be more than 2 or 3 times the size of the desired reproduction. Wherever possible two or more drawings should be grouped to reduce the number of cuts required. In such groups of drawings, or in large drawings, full use of the space available should be made; the ratio of height to width should conform to that of a journal page (5½×7½ in.) but allowance must be made for the captions. **The original drawings and one set of clear copies (e.g. small photographs) are to be submitted.**

Photographs.—Prints should be made on glossy paper, with strong contrasts. They should be trimmed so that essential features only are shown and mounted carefully, with rubber cement, on white cardboard, with no space between those arranged in groups. In mounting, full use of the space available should be made. **Photographs are to be submitted in duplicate;** if they are to be reproduced in groups one set should be mounted, the duplicate set unmounted.

REPRINTS

A total of 50 reprints of each paper, without covers, are supplied free. Additional reprints, with or without covers, may be purchased at the time of publication.

Charges for reprints are based on the number of printed pages, which may be calculated approximately by multiplying by 0.5 the number of manuscript pages (double-space typewritten sheets, 8½×11 in.) and including the space occupied by illustrations. Prices and instructions for ordering reprints are sent out with the galley proof.

Contents

James Trotter—Steric inhibition of resonance. IV. Further spectroscopic studies of 9-nitroanthracene and 9,10-dinitroanthracene - - - - - 1009

D. H. Ball and G. A. Adams—A mannan produced by *Bacillus polymyxa* - 1012

D. J. Ball, A. E. Flood, and J. K. N. Jones—5,6-Dideoxy-L-arabino-hexose (5-deoxy-5-C-methyl-L-arabinose) - - - - - - - - - - - - - 1018

R. F. Platford and J. W. T. Spinks—Irradiation of aqueous chloral hydrate with Sr^{90}–Y^{90} beta rays - - - - - - - - - - - - - - - - 1022

N. A. Khan—Some specific features in autoxidation of methyl linoleate - 1029

Everett Thiele and David J. Wilson—A comparison of Slater's theory of unimolecular reactions with experimental data - - - - - - - - 1035

E. von Rudloff—The wax of the leaves of *Picea pungens* (Colorado spruce) - 1038

A. V. Robertson and Léo Marion—The biogenesis of alkaloids. XXI. The biogenesis of (−)-homostachydrine and the occurrence of trigonelline in alfalfa - - - - - - - - - - - - - - - - - - - 1043

M. Jackson and L. D. Hayward—The action of sodium iodide on *O-p*-toluenesulphonyl-1,4;3,6-dianhydrides of D-mannitol, D-glucitol, and L-iditol - 1048

Jacques Collin—Ionization potentials of methylamines and ethylamines by electron impact - - - - - - - - - - - - - - - - - 1053

R. J. Abraham and H. J. Bernstein—The proton resonance spectra of furan and pyrrole - - - - - - - - - - - - - - - - - - 1056

Alberto Breccia and Léo Marion—The biogenesis of alkaloids. XXII. Some intermediate steps in the transformation of tryptophan to gramine - 1066

D. J. McCaldin and Léo Marion—The nature of pyraconitine - - - - - 1071

A. H. Turner and R. J. Cvetanović—Isotopic and hot radical effects in the reaction of hydrogen atoms with ethylene - - - - - - - - - 1075

J. F. Henderson and C. A. Winkler—The reaction of potassium persulphate with thioglycolic acid in aqueous solution - - - - - - - - - 1082

M. J. Cabell—The purification, determination, and neutron capture cross section of actinium-227 - - - - - - - - - - - - - - - - 1094

K. K. Georgieff and A. Dupré—Preparation and infrared spectra of divinyl sulphide, 2-methyl-1,3-thioxolane, and 1,4-thioxane - - - - - - 1104

Janet S. Merritt, J. G. V. Taylor, and P. J. Campion—Self-absorption in sources prepared for 4π beta counting - - - - - - - - - - 1109

Notes:

M. Joyce Smith—Independence in hydrolysis of condensed phosphates - 1115

Maria Przybylska and Léo Marion—The X-ray structure analysis of demethanol aconinone hydriodide trihydrate - - - - - - - - 1116

K. H. Kossmann—2,4-Diamino-2-methylpentane diisocyanate and its methyl urethane - - - - - - - - - - - - - - - - - 1118

S. Brownstein—A simple thermostated probe assembly for high resolution nuclear magnetic resonance spectra - - - - - - - - - - - 1119

Francis L. Chubb and Jacqueline Nissenbaum—Some hypoglycemic thiadiazoles - - - - - - - - - - - - - - - - - - - 1121

Robin M. Hochstrasser—Note on the photooxidation of tetramethylrubrene 1123

Corrections - i

Printed in Canada *by* University of Toronto Press